Anticipating the Environmental Risk Assessment of crops modified to enhance or preserve yield.

August 2010

COGEM Report CGM 2010-05

Patrick L.J. RÜDELSHEIM & Greet SMETS
PERSEUS BVBA

PERSEUS

Biosafety and Biotechnology Regulatory Services

Ordering information

COGEM report No CGM 2010-05
E-mail: info@cogem.net
Phone: +31-30-274 2777
Fax: +31-30-274 4476
Postal address: Netherlands Commission on Genetic Modification,
P.O. Box 578, 3720 AN Bilthoven, The Netherlands
Internet Download as pdf-file: http://www.cogem.net → publications → research reports

When ordering this report, always mention title and number.

Advisory Committee:

The authors gratefully acknowledge the members of the advisory committee for the valuable discussions and patience.
Chair: Gerco Angenent (Plant Research International)
Members: Tom de Jong (Leiden University)
Bert de Boer (VU University Amsterdam)
Boet Glandorf (GMO Office)
Fenne Koning (COGEM secretariat)

Disclaimer

Table of contents

Abbreviations

Names of individual genes and proteins, that are mentioned in the text, are not included in this list

ABA	Abscisic acid
ABF	ABA-responsive element binding factor
ABRE	ABA-responsive element, promoter element
ADP	Adenosine diphosphate
AHTEG	Ad Hoc Technical Expert Group (Cartagena Protocol)
APHIS	Animal and Plant Health Inspection Service
AREB	ABA-responsive element-binding
AT-hook	DNA-binding motif consisting of a conserved, palindromic, core sequence of proline-arginine-glycine-arginine-proline
ATP	Adenosine triphosphate
BASF	Badische Anilin- und Soda-Fabrik
bHLH	Basic helix-loop-helix; DNA binding motif characterized by two α-helices connected by a loop
BL	Brassinolide
BR	Brassinosteroid
bZIP	Basic leucine zipper, DNA binding domain and dimerization domain
CaMV	Cauliflower Mosaic Virus
CBF	C-repeat-binding factor
CBI	Confidential Business Information
CDC	Cell Division Cycle
CDK	Cyclin-dependent kinase
CGB	Commission du Génie Biomoléculaire, France
CGIAR	Consultative Group on International Agricultural Research, USA
CIAT	Centro Internacional de Agricultura Tropical, Colombia
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico
CNB	Comisión Nacional de Bioseguridad, Spain
COGEM	Commissie Genetische Modificatie
COP-MOP	Conference of the Parties serving as the meeting of the Parties to the (Cartagena) Protocol
CSP	Cold-shock protein
CsVMV	Cassava vein mosaic virus
CYC	Cyclin
DELLA proteins	Proteins with the amino acid sequence DELLA in their primary structure; repressors of transcription.
DIR	Dealing involving intentional release; Autralian equivalent of ‘deliberate release’
DNA	Deoxyribonucleic acid
DP	Dimerization Partner
DRE	Dehydration-responsive element; promoter element
DREB	Dehydration-responsive element binding
E x G	Environment – genotype interaction
E2F	TF family involved in the cell cycle regulation and synthesis of DNA
EFSA	European Food Safety Authority
ERA	Environmental Risk Assessment
EREBP	Ethylene-responsive element binding proteins
ERF	Ethylene-responsive element binding factor
EU	European Union

F6P	Fructose 6-phosphate
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration, USA
FPI	Floral Pathway Integrators
FR	Far red light
FSANZ	Food Standards Australia New Zealand
G1/S	Transition in the cell cycle from the 'gap'1 phase to the phase of DNA synthesis
G1P	Glucose-1-phosphate
G2/M	Transition in the cell cycle from the 'gap'2 phase to mitosis
G6P	Glucose-6-phosphate
GA	Gibberellic Acid
GG	Genetisch Gemodificeerd
GM	Genetic Modification – Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
GRAS	Family of proteins named after GAI, RGA and SCR.
HD	Homeodomain, DNA and RNA binding domain consisting of a 60-amino acid helix-turn-helix structure in which three alpha helices are connected by short loop regions
HD-Zip	Homeobox-leucine zipper domain
HSP	Heat-shock protein
IAA	Indole-3-acetic acid
IITA	International Institute of Tropical Agriculture, Nigeria
IRRI	International Rice Research Institute, the Philippines
LMO	Living modified organism
LRR	Leucine rich repeat
MADS	TF family named after its first characterized members: MCM1, AGAMOUS, DEFICIENS, SRF (serum response factor)
MAP	Mitogen-activated protein kinases
MMA	Ministerio de medio Ambiente y medio rural y marino, Spain
MRB	Milieurisicobeoordeling
MYB	TF family named after avian myeloblastosis virus oncogene
MYC	TF family named after myelocytomatosis oncogene
NAC	TF family named after the proteins NAM, ATAF and CUC
NAD+	Nicotinamide adenine dinucleotide, oxidized form, coenzyme
NADH	Nicotinamide adenine dinucleotide, reduced form, coenzyme
NADP+	Nicotinamide adenine dinucleotide phosphate, oxidized form
NDP	Nucleoside diphosphate
NF-Y	Nuclear factor Y family of TFs; active as heterotrimeric complexes consisting of NF-YA, NF-YB, and NF-YC subunits
NRC	The US National Research Council
NTO	Non-target organism
NTP	Nucleoside triphosphate
NUE	Nitrogen Use Efficiency / Nutrient Use Efficiency
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator, Australia
PHY	Phytochrome
PPi	Pyrophosphate
QTL	Quantitative Trait Locus
R	Red light
RARM	Risk Assessment and Risk Management

RNA	Ribonucleic Acid
RNAi	Ribonucleic Acid interference
ROS	Reactive oxygen species
S6P	Sucrose-6-phosphate
SAM	Shoot apical meristem
SET	A 130-amino acid conserved sequence motif; protein family of histone lysine methyltransferases
SNIF	Summary Notification Information Format
SWG	Sub-Working Group
T6P	Trehalose-6-phosphate
TCP	TF family named after its first characterized members: TB1, CYC and PCFs
TF	Transcription Factor
UDP	Uridine diphosphate
UDPG	Uridine diphosphoglucose
USA	United States of America
USDA	United States Department of Agriculture
VROM	Ministerie Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer; (the Ministry of Housing, Spatial Planning and the Environment)
WRKY	Class of proteins with a 60 amino acid domain, with the N-terminal conserved amino acid sequence WRKYGQK and a C-terminal zinc-finger-like motif
WUE	Water Use Efficiency
ZF-HD	Zinc-finger homeodomain, containing a 60-amino acid DNA-binding domain and a zinc-finger motif for dimerization

Summary

Tomorrow's crops will be required to yield more, to perform in spite of less favourable growing conditions and to limit the environmental impact of cultivation. Several breeding approaches are being pursued, including the use of genetic modification (GM). Already a large number of field trials include GM traits that target enhancing and securing yield and the first products are nearing market introduction. In the USA, Canada, the EU, Australia, New Zealand and the Philippines Monsanto has submitted a dossier for commercialization of the first drought tolerant maize that was developed in collaboration with BASF. ArborGen is seeking permission to commercialize frost tolerant eucalypts in the USA.

Some authors suggested that there might be specific issues associated with the introduction of *e.g.* a stress tolerance trait. They assume that an increased tolerance to stress factors such as drought, high temperatures, salt, water lodging, low temperatures or frost may influence the GMO's fitness. Similarly, speed of germination and growth, enhanced photosynthesis, better use of soil minerals are linked to potential effects on the plant's reproduction, invasiveness and persistence. Yet, it is not always clear on what basis such assumptions are based and to what extent these issues are specific or different for GM plants with traits that target enhancing or securing yield.

Aim of this study
Anticipating the presentation of regulatory files covering field trials and commercial introduction of plants with yield enhancing and yield securing traits, this study was targeted to:
- Provide a comprehensive outlook on the developments and expectations in relation to the deployment of such traits; and
- Identify aspects that will be important for the Environmental Risk Assessment (ERA) of plants incorporating these traits and that are different from those already known for first generation GMOs.

Given the vast area to be covered, the scope of the project was further determined by:
- Focussing on those strategies that could be considered novel in terms of the ERA;
- Limited to those that were in development or in a pre-commercial stage; and
- Restricting to those for which relevant information such as regulatory documents, patent applications and scientific publications was available.

Inventory of technologies
Yield enhancement can be achieved in very diverse ways of modifying basic functions of a plant, such as growth rate, photosynthesis, plant architecture, nutrient uptake and metabolism, ratio above-ground to below-ground biomass, transition to flowering, seed set, harvest index, source-sink relationship etc. Different phenomena may be influenced by the same genetic elements, plant compounds and mechanisms; each being a potential target for a research strategy. The identified strategies include the use of cell cycle genes, plant hormones (gibberellins, brassinosteroids, auxins, cytokinins) flowering genes, sugar-signalling (trehalose pathway, hexoses), transcription factors and other yield enhancing genes.

On the other hand, yield can be secured by ensuring that the plant has the capacity to perform even in sub-optimal conditions. In this study only abiotic stress tolerance was considered. Primary stresses, such as drought, salinity, cold, heat and chemical pollution often result in similar effects, and cause cellular damage and secondary stresses, such as osmotic and oxidative stress. The initial stress signals (*e.g.* osmotic and ionic effects, or temperature, membrane fluidity changes) trigger the downstream signalling process and transcription controls which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes. Attempts to obtain plants with an increased abiotic stress tolerance involved modification of genes in either of these processes.

Based on the review of this inventory, some general considerations were formulated:

- These strategies are typically elaborating on plant-own mechanisms and/or require influencing complex biochemical pathways. Often genes are taken from plants, sometimes from the same species.

- Stress responses in plants show high levels of complexity and redundancy at the perception, signalling and expression levels with cross regulation ("cross-talk") between stress pathways and overlapping functions between stress metabolites and stress proteins in different stresses. Molecular approaches vary from expressing new functions, to over-expression and silencing of plant-own functions.

- Especially when influencing factors that intervene in different plant processes (*e.g.* plant hormones or signalling functions) complex effects are observed. In consequence, the inserted function is often controlled by a promoter that limits the expression to specific plant parts, development stages or growing conditions (*e.g.* when the stress factor is present).

- There are very few reports on strategies that both secure yield under stress conditions and enable yield improvement under optimal conditions.

- Any engineered strategy will act in addition to and/or in interaction with the already available mechanisms that will be triggered simultaneously.

- The large diversity of targets and strategies makes referring to the trait as abiotic stress tolerance misleading. Typically only a very specific stress response is targeted.

- Irrespective of sometimes complex changes, the resulting plants were still considered as basically comparable to the recipient organism. While the architecture may have changed (*e.g.* dwarf growth, increased number of tillers) the overall aspect of the plant as well as its developmental characteristics were maintained.

Specific aspects of the ERA

In the second part of the study, a synthesis is proposed summarizing those aspects of an ERA that might require specific attention when evaluating GM plants with yield enhancing or securing traits.

- Some of the new approaches influence mechanisms inherent to the plant, rather than introducing completely new functions. While also other traits may be based on influencing plant-own metabolic systems (*e.g.* modified composition), this may require adapting the standard product characterization paradigm.

- While the introduced sequences may be limited, the resulting phenotypic changes may be complex. Some traits influence different reaction pathways or can effect different responses. Any modification that could have an effect on the environmental impact needs to be identified at the start of the ERA. Given the complexity of some effects, it will be challenging in some cases to get a complete description. Still, it remains feasible to conduct an ERA on the basis of a complex phenotype. The starting point is that all significant differences with non-modified plants are well characterized.

- Product characterization is largely based on a comparative assessment. In the case of stress tolerance special care is required to identify receiving environments (as these may be broadened based on the trait), comparators and baselines (as these plants will by definition behave differently under stress exposure), interaction with other stress factors and variation that may be caused by controlled expression.

- The potential for increased "weediness" of the GM plant is a central -yet badly defined- concern. Many yield enhancement traits show similarity with characteristics included in Baker's list of characteristics common to weeds and giving the plant a selective advantage. On the other hand, there are also yield enhancing traits that contribute to further domestication, often leaving the plant with a disadvantage in natural habitats.

- Adding one trait to an otherwise non-invasive, non-persisting and non-disseminating crop will not turn it into a weed. High yielding plants are usually limited by a range of factors, such as a higher nutrient requirement, soil and light conditions, etc. Nevertheless, it can be anticipated that over time several yield enhancing and securing traits may be combined. Due to a lack of our understanding of the environmental conditions that control species, it remains at this moment impossible to predict which combination of traits will result in an ecological release.

- Possible advantages of stress tolerance are often balanced with costs associated with the expression of the functions. Stress tolerance genes constitutively expressed will have a higher energy cost compared to tissue-specific or conditionally expressed genes, giving a selective disadvantage.

- Depending on the compatibility of the plant with wild relatives, the introduced traits might introgress in wild populations. In most cases the wild plant populations are already highly adapted to varying environmental conditions, including several stress factors.

- Very often the genes and functions are already abundantly present in the environment. The majority is derived from plants, often food plants or from the recipient plant itself. As a consequence toxicity and allergenicity (*e.g*. via accidental consumption, contact) will in most cases not be an issue. Nevertheless this requires a case-by-case analysis, as some plant mechanisms involve, trigger or may mask the synthesis of anti-nutritional or allergenic compounds.

- Finally, expanding the growing area of the crop potentially could have -just like any other form of agriculture- an important impact on the local ecosystems.

Although each trait is specific and it is impossible to make general statements, this analysis concludes that based on the information that is available; all aspects can be covered in the existing structure of the ERA.

Samenvatting

De gewassen van de toekomst zullen meer moeten opbrengen, ook bij minder gunstige groeiomstandigheden, en hun teelt zal minder moeten drukken op het milieu. In de veredeling worden verschillende benaderingen in overweging genomen, genetische modificatie incluis. Nu al loopt er een groot aantal veldproeven waarin kenmerken voor opbrengsttoename en –veiligstelling worden getest en de eerste producten zullen weldra op de markt verschijnen. In de VS, Canada, de EU, Australië, Nieuw-Zeeland en de Filippijnen heeft Monsanto een dossier ingediend voor commercialisering van de eerste droogtetolerante maïs die werd ontwikkeld in samenwerking met BASF. Eveneens in the VS heeft ArborGen een kouderesistente eucalyptus aangemeld voor markttoelating.

Sommige auteurs suggereren dat er specifieke kwesties aan de orde zijn bij de introductie van bv. droogtetolerantie. Zij gaan ervan uit dat een verhoogde weerstand aan stressfactoren zoals droogte, hoge temperaturen, verzilting, overstroming, koude of vorst de fitness van GGO's kan beïnvloeden. Op de zelfde wijze worden kiem- en groeisnelheid, verhoogde fotosynthese, betere benutting van mineralen gekoppeld aan mogelijke effecten op de reproductie van de plant, aan invasiviteit en aan persistentie. Toch is het niet altijd duidelijk op welke basis die aannames worden gedaan en in welke mate deze aspecten specifiek of verschillend zijn voor GG-planten met kenmerken die beogen opbrengsten te verhogen of veilig te stellen.

Doel van dit rapport
Vooruitlopend op de te verwachten vergunningaanvragen voor veldproeven en commercialisering van gewassen met opbrengstbevorderende en -handhavende eigenschappen, is het doel van dit rapport om:
- te voorzien in een bevattelijk overzicht van de ontwikkelingen en verwachtingen op het gebied van het aanwenden van deze kenmerken;
- die aspecten te identificeren die van belang zullen zijn in de milieurisicobeoordeling (MRB) van planten waarin deze eigenschappen werden ingebouwd en die verschillen van deze die al bekend zijn van de eerste generatie GGO's.

Wetende dat het toepassingsgebied zeer breed is, werd de reikwijdte van het project verder bepaald door:
- de aandacht toe te spitsen op die strategieën die als nieuw kunnen beschouwd worden in termen van een MRB;
- de toepassingen te beperken tot die, die in de fase van ontwikkeling of precommercialisering zitten; en
- het aantal werkwijzen te limiteren tot die, waarvoor relevante informatie zoals kennisgevingen en risicoanalysen, patentaanvragen en wetenschappelijke publicaties beschikbaar zijn.

Inventariseren van technologieën
De opbrengst kan op verschillende manieren verhoogd worden door de basisfuncties van de plant te wijzigen zoals groeisnelheid, fotosynthese, plantenarchitectuur, opname en metabolisme van voedingsstoffen, de verhouding bovengrondse tot ondergrondse biomassa, overgang van de vegetatieve naar de generatieve fase, zaadzetting, oogstindex, 'source-sink' relatie enz. Verschillende fenomenen kunnen beïnvloed worden door soortgelijke genetische elementen, inhoudstoffen en mechanismen; die elk op zich een mogelijk doelwit zijn voor een onderzoeksstrategie. De strategieën die werden geïdentificeerd omvatten het gebruik van celcyclusgenen, plantenhormonen (gibberellinen, brassinosteroïden, auxinen, cytokininen), genen i.v.m. de bloei en suikersignalering (trehaloseroute, hexoses), transcriptiefactoren en andere opbrengstbevorderende genen.

Aan de andere kant kan opbrengst worden veiliggesteld door er voor te zorgen dat de plant in staat is ook in suboptimale condities te gedijen. In deze studie wordt alleen abiotische stresstolerantie besproken. Primaire stressfactoren, zoals droogte, verzilting, koude, hitte en chemische pollutie induceren dikwijls gelijkaardige reacties. Ze veroorzaken schade aan de cel

en veroorzaken secundaire stress zoals osmotische en oxidatieve stress. De initiële stresssignalen (bv. osmotische en ionisatie effecten, of temperatuursveranderingen en veranderingen in de structuur van membranen) brengen verder stroomafwaarts een proces van signaaltransductie en controle door transcriptiefactoren op gang die specifieke mechanismen activeren om het celmilieu te herstellen en proteïnen en membranen te beschermen of te herstellen. Pogingen om planten te maken met een verhoogde abiotische stresstolerantie grijpen in op de genen die betrokken zijn in dergelijke processen.

Uitgaande van dit overzicht kunnen enkele algemene bedenkingen worden geformuleerd:

- Deze strategieën bewerken typisch planteigen mechanismen en/of vereisen dat complexe biochemische routes worden beïnvloed. Dikwijls is de oorsprong van de genen plantaardig, soms zijn ze zelfs afkomstig van de zelfde soort.

- De stressrespons in planten vertoont een hoge complexiteitsgraad en redundantie ter hoogte van de perceptie, signalering en expressieniveaus met wederzijdse regulering (“cross-talk”) van stressroutes en overlappende functies tussen stressmetabolieten en stressproteïnen bij de verschillende soorten stress. Moleculaire benaderingen variëren van het tot expressie brengen van nieuwe functies tot het verhogen van de expressie of uitschakelen van planteigen functies.

- Zeker wanneer beïnvloedende factoren tussenkomen in verschillende plantenprocessen (bv. plantenhormonen of signaalfuncties), worden complexe effecten waargenomen. Daarom wordt het binnengebrachte gen dikwijls onder controle van een promoter geplaatst die de expressie beperkt tot specifieke plantendelen, ontwikkelingsstadia of groeiomstandigheden (bv. wanneer de stressfactor aanwezig is).

- Er zijn zeer weinig rapporten over strategieën die zowel de opbrengst handhaven onder stresscondities en tegelijkertijd de opbrengst verhogen onder optimale omstandigheden.

- Gelijk welke uitgewerkte strategie zal additioneel werken op en/of in interactie gaan met de reeds aanwezige mechanismen die dan tegelijkertijd gestimuleerd worden.

- De grote verscheidenheid aan doelen en strategieën maakt dat verwijzen naar een eigenschap als abiotische stresstolerantie misleidend is. Meestal wordt alleen een specifieke stressrespons aangepakt.

- Onafhankelijk van de soms complexe wijzigingen, worden de gemodificeerde planten nog steeds beschouwd als fundamenteel vergelijkbaar met de recipiënte soort. Hoewel het voorkomen kan veranderd zijn (bv. dwerggroei, toegenomen aantal stoelen) blijven het algemene uiterlijk van de plant zowel als zijn ontwikkelingskarakteristieken behouden.

Specifieke aspecten van de MRB

In het tweede deel van het overzicht wordt een synthese gemaakt die aspecten van een MRB samenvat die mogelijk speciale aandacht vereisen bij de evaluatie van GG-planten met kenmerken voor opbrengsttoename en –veiligstelling.

- Sommige van de nieuwe genetische technieken beïnvloeden mechanismen inherent aan de plant, eerder dan een compleet nieuwe functie te introduceren. Hoewel ook andere kenmerken kunnen gebaseerd zijn op het beïnvloeden van planteigen metabolische systemen (bv. een gewijzigde samenstelling van inhoudsstoffen), is mogelijk dat ze een aanpassing vereisen van de aanpak van de standaard productkarakterisering.

- Hoewel de ingebrachte sequenties mogelijk beperkt zijn, kunnen de resulterende fenotypische veranderingen complex zijn. Dit is te wijten aan het feit dat sommige kenmerken verschillende reacties kunnen beïnvloeden of verschillende effecten kunnen veroorzaken. Gelijk welke modificatie die een effect op het milieu kan hebben, moet bij het begin van de MRB worden geïdentificeerd. Toch zal het, gegeven de complexiteit van sommige effecten, in sommige gevallen een uitdaging zijn om een complete beschrijving te

verkrijgen. Het is wel degelijk mogelijk een MRB uit te voeren op basis van een complex fenotype. Het uitgangspunt is dat alle significante verschillen met niet-gemodificeerde planten goed gekarakteriseerd zijn.

- Productkarakterisering wordt voor een groot deel gebaseerd op een vergelijkende evaluatie. In het geval van stresstolerantie moet speciaal aandacht worden besteed aan het identificeren van het milieu waarin de GG-plant zal worden geïntroduceerd (immers, het normale teeltgebied kan worden uitgebreid naargelang het kenmerk), aan het geschikte vergelijkingsmateriaal en de basislijn (omdat die planten zich per definitie verschillend zullen gedragen onder stresssituaties), aan interacties met andere stressfactoren en aan variatie die kan worden veroorzaakt door gecontroleerde expressie.

- Of een GG-plant mogelijkerwijs meer op een onkruid gaat lijken –hoewel dit niet goed gedefinieerd- is een belangrijke zorg. Het opvoeren van het opbrengstpotentieel en de verbetering van de stresstolerantie van gewassen zou de GG-planten direct of indirect een selectievoordeel kunnen opleveren. Vele opbrengstbevorderende kenmerken vertonen gelijkenis met de eigenschappen die staan opgesomd in de lijst van Baker om gewone onkruiden te beschrijven. Aan de andere kant zijn er ook opbrengstverhogende eigenschappen die duidelijk in het verlengde liggen van domesticatie.

- Eén eigenschap toevoegen aan een verder niet-invasief, niet-persistent en zich niet-verspreidend gewas zal het niet veranderen in een onkruid. Hoogopbrengende planten zijn gewoonlijk ingeperkt door een reeks factoren zoals een grotere bemestingsbehoefte, bodem- en lichtcondities enz. Niettemin moet er rekening mee worden gehouden dat in de loop van de tijd verschillende kenmerken die de opbrengst verhogen of handhaven kunnen worden gecombineerd. Omdat we nog te weinig inzicht hebben in de omgevingsfactoren die soorten controleren, is het op dit moment onmogelijk te voorspellen welke combinaties van kenmerken zullen resulteren in een plant die aan de huidige omgevingscontroles ontsnapt.

- Mogelijke voordelen van stresstolerantie worden vaak afgewogen tegen de kost die samenhangt met de expressie ervan. Stresstolerantiegenen die constitutief tot expressie worden gebracht, zullen een hogere energiekost hebben vergeleken met genen die weefselspecifiek of conditioneel tot expressie komen, en daardoor een selectief nadeel ondervinden.

- Afhankelijk van de compatibiliteit van de plant met wilde verwanten, kunnen de ingebracht eigenschappen inkruisen in wilde populaties. In vele gevallen zijn de wilde plantenpopulaties al in grote mate aangepast aan wisselende milieuomstandigheden, inclusief stressfactoren, en zal de bijkomende functie weinig effect hebben.

- Vaak zijn de genen en bijbehorende functies al wijdverspreid in het milieu. De meerderheid is afkomstig van planten, dikwijls voedselgewassen of van de recipiënte plant zelf. Als gevolg daarvan zal toxiciteit en allergeniciteit (bv. via accidentele consumptie, contact) in de meeste gevallen geen punt zijn. Toch moet dit geval-per-geval bekeken worden want sommige plantenmechanismen zijn betrokken bij de aanmaak van anti-nutritionele of allergene stoffen, kunnen aanzetten tot zulke aanmaak of kunnen de aanmaak overschaduwen.

- Tot slot heeft de potentiële uitbreiding van het teeltareaal van het gewas –net als elke andere vorm van landbouwbedrijven- een belangrijke impact op het locale ecosysteem.

Hoewel elk kenmerk specifiek is en het onmogelijk is om algemene uitspraken te doen, leert deze analyse dat voortbouwend op de beschikbare informatie, alle aspecten kunnen worden behandeld in de bestaande structuur van de MRB.

1 Project objectives and methodology

1.1 Background

The stakes to develop improved food and feed crops are very high: to satisfy the rapidly increasing world population a greater-than-ever quantity of high quality food has to be foreseen, while limitations on land-use, scarcity of inputs like suitable water, the desire to preserve natural resources and the changing climate impose unequalled challenges. Tomorrow's crops will be required to yield more, to perform in spite of less favourable growing conditions and to limit the environmental impact of cultivation.

One of the approaches to broaden crop improvement is genetic modification (GM). First-generation GM technology in crops involves altering some aspect of production, leaving the end product identical to that produced by a conventional crop. Early examples include crops modified to carry a tolerance to a particular herbicide and/or a defence mechanism against certain insect pests. A range of products in different crops has been the subject of safety assessments and they are cultivated and traded around the globe.

These first applications contribute to securing agricultural produce by protecting the crops against a specific influencing factor (*i.e.* weeds and insect pests respectively). There are many more factors that have an impact on agronomic yield. Potentially each of these factors represents a target for developing strategies for enhancing and securing yield. In consequence the application of GM technology has expanded to include other yield related traits such as traits associated with plant development or stress tolerance.

Already a large number of field trials include traits that target enhancing and securing yield. The first products are nearing market introduction. In the USA, Canada, the EU, Australia, New Zealand and the Philippines Monsanto has submitted a dossier for marketing of the first drought tolerant maize that was developed in collaboration with BASF; ArborGen is seeking permission to commercialize frost tolerant eucalypts in the USA.

1.2 Objectives

Anticipating the presentation of regulatory applications covering field trials and commercial introduction of plants with yield enhancing and yield securing traits, the information presented and analysed in this report was expected to:

- Provide a comprehensive outlook on the developments and expectations in relation to the deployment of such traits
  This objective comprehended the establishment of an inventory of advanced research and development of such traits in plants, with special emphasis on those applications that could be expected to result in products. Based on a literature review possible technical approaches and their specific features were to be summarized and structured.
- Identify aspects that will be important for the Environmental Risk Assessment (ERA) of plants incorporating these traits.
  Given the identified range of techniques, a critical review of issues relevant for the ERA approach had to be presented. This needed to be coherent with the European Directive 2001/18/EC, elaborated in additional guidance documents, and implemented in the Netherlands through the GMO Decree.

1.3 Methodology

This study was conducted by Dr. P. Rüdelsheim and Dr. Ir. G. Smets of Perseus BVBA. The realization of this project was under the supervision of an Advisory Committee composed of:

Chair: Gerco Angenent (Plant Research International)
Members: Tom de Jong (Leiden University)
Bert de Boer (VU University Amsterdam)

Boet Glandorf (GMO Office)
Fenne Koning (COGEM secretariat)

Based on an initial survey of different technologies, a framework was established to specify the scope of this study. The scope was confirmed by the Advisory Committee. Subsequently a detailed review of technologies was performed based on scientific review papers, searches in literature databases, patent applications as well as information from authorities on introductions in the environment for field trials and commercialization. No distinction was made between research on model crops or developments with a commercial intention during the searches.

This survey resulted in a listing of possible strategies, their characteristics, the underlying mechanism, if known, and the developmental phase they were in at the time of the survey. Where relevant, interactions with other metabolic pathways that could indicate multiple effects were highlighted.

In order to analyse possible impacts, technologies were grouped according to the targeted function. Where strategies for one target differ significantly and that difference was expected to influence the ERA, subgroups were maintained based on mechanism or metabolic pathway.

In parallel a review of the legal framework was carried out. The scope was determined in consultation with the Advisory Committee. Official documents and technical guidance notes were accessed directly from the official sources. A search was done to verify precedents of ERAs performed by other authorities on releases of this type of GM plants.

When structuring the analysis for specific ERA elements the approach required by the European legislation and guidance documents was followed as much as possible. The first part reviews any specific aspect related to poduct characterization which is the starting point for any ERA. Subsequently all the topics listed in Annex II.D2 of Directive 2001/18/EC are covered. This section of the Directive specifies for GM higher plants (GMHP) the following points on which information is required, as appropriate, in order to draw conclusions on the potential environmental impact from the release or the placing on the market of GMOs:

- Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.
- Any selective advantage or disadvantage conferred to the GMHP.
- Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.
- Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).
- Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.
- Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).
- Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
- Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
- Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

The collection of information was concluded on 15 March 2010.

1.4 Justification for determining the scope of the project

A broad variety of plant characteristics is susceptible to influence yield under certain circumstances. In order to determine the scope of this study an initial framework for positioning potential GM applications was prepared

When considering ways of increasing and securing crop produce several approaches can be envisaged:

- Increasing the production potential ("intrinsic yield potential"):
  - In terms of harvestable yield;
  - In terms of usable product (for instance by increasing the ratio of the desired end-product in the harvested material).
- Improving the realization of the maximum potential:
  - By allowing plants to better utilize resources in order to satisfy their needs;
  - By limiting the impact of stress factors.
- increasing the cultivation area:
  - By replacing other crops on the land used for agriculture;
  - By expanding into marginal land and other land not yet used for agriculture.

Some strategies will support possibly different targets. For instance a drought tolerance trait will make plants less sensitive to reduced water inputs and may allow expansion of the growing area into marginal land or into regions where the crop was not planted before. The grouping of the different strategies is pictured in Figure 1.

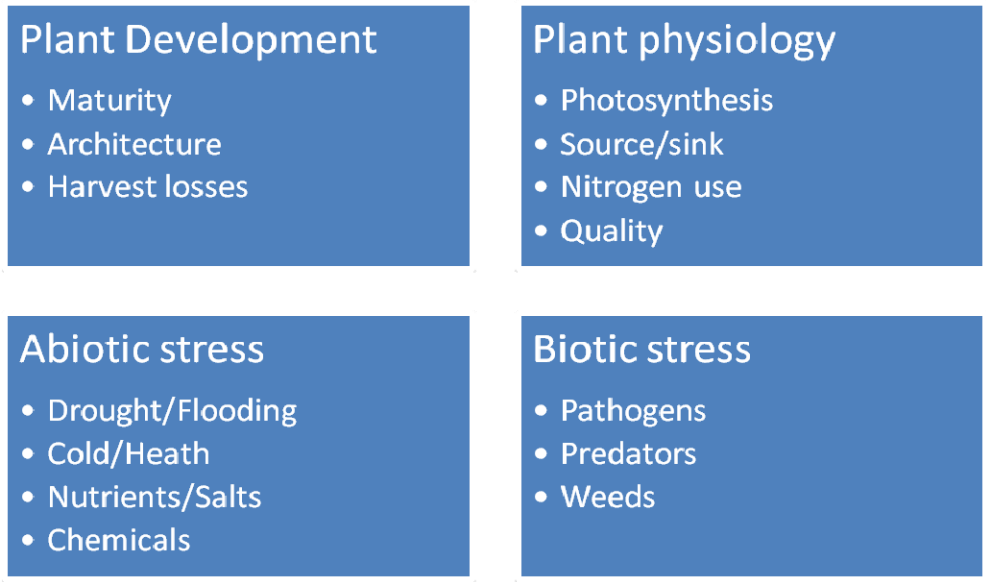

Fig.1 Grouping of strategies related to yield enhancement and preservation

1.4.1 Exclusion criteria 1: Novelty for ERA

At the onset of the project it was determined to focus on those strategies that could be considered novel in terms of ERA. In consequence the following strategies were excluded from the study:

- Quality modifications:
  Several applications for products with modified composition have been handled in the past (*e.g.* commercial applications in EU include potato event EH92-527-1 (Amflora) and high oleic acid soybean event 305423; field trial applications in EU include oilseed rape with improved oil composition).
- Biotic stress tolerance traits:

- Pathogens and pests: early applications already included insect pest resistance. The ERA associated with these applications has been documented and further work on disease resistances has been based on these paradigms.
- Herbivores: there is little information available on introducing tolerance traits to large herbivores such as rabbits, deer etc.
- Weeds: herbicide tolerance has effectively been introduced as an extension of using crop protection products for weed control. Strategies that relate to plant architecture were included in the scope of this review, irrespective if they could also affect competition with weeds.

Although these GM strategies were excluded from the scope, some references are included whenever it was expected that another GM strategy may have side-effects that could lead to *e.g.* biotic stress tolerance. Although the expression of these side-effects might not be at a level to be exploited commercially, they are an integral part of the effects that need to be considered in an ERA.

1.4.2 Exclusion criteria 2: Stage of development

The collection of techniques was further limited to those that were in development or pre-commercial stage. The indication was provided to limlit the scope to applications with for which applications for field trials or marketing (including importation as products) in the EU and the Netherlands can be expected in the coming years (until 2015).

This selection was based on information of applications for field trials and commercial release was collected. However, only data from the 5 most recent years are discussed, the rationale being that genes that were not reported in this period probably did not deliver the proof of concept or were abandoned for other reasons.

1.4.3 Exclusion criteria 3: Availability of relevant information

Data were collected from the European part B applications, from US USDA notifications and Australian applications. Australian and EU field trial applications provide an insight in the result of the ERA. In the US no separate ERA is required for notifications and most permit applications (competent authority to decide). Only permits for commercial use contain ERA conclusions. Yet, they provide a good outlook as most developers have initial field trials in North America.

Several constraints make it difficult to have a clear and complete picture of the ongoing research, especially in the US:

- Genes that code for certain traits are often not indicated due to confidential business information (CBI) claims. For example, one company applied 24 times for field trials in the US with plants with an increased germination rate without disclosing the nature of the genes (see tables Annex 3). Also in other countries the designation and function of the genes of interest in a trial application are sometimes not disclosed when private companies are involved. Only for trials applied for by research institutes more information about the inserted genes is communicated. Therefore the analysis tends to be biased towards developments by public research institutes. In order to compensate, patent applications of private companies, as far as publically available, were consulted.
- One application may concern a long list of genes. It is not known which genes are combined in one event or which genes are trialled individually. It is even not always clear whether sense or antisense constructs are used.
- Regulatory elements such as promoters, enhancers, targeting sequences or terminators are often not described.
- Only a classification is disclosed, like in the maize 06-019-04R and soybean 06-019-02R and 06-019-01R permit applications, where genes for MYB transcription factors (TF) are utilized. It is not disclosed which process will be influenced.
- Finally, often a list of genes and a list of traits/phenotypes are given without clarifying what action is expected from which gene.

Tables with summary data on field trials concerning yield enhancement and abiotic stress tolerance for EU, Australia and USA are presented in respectively Annex 1, Annex 2 and Annex 3.

Ongoing evaluations for commercialization are:

- Drought resistant maize, transformation event MON 87460 in USA (FDA: food & feed, APHIS 09-055-01p, environment), in Canada[1] (food, feed & environment), in EU (EFSA-GMO-NL-2009-70: food & feed); Australia/New Zealand (FSANZ Application A1029: food) and in the Philippines[2] (food & feed).
- Freeze tolerant eucalyptus transformation event ARB-FTE1-08 in USA (APHIS 08-366-01p).

These will be discussed in the chapter on abiotic stress tolerance.

[1] http://www.inspection.gc.ca/english/plaveg/bio/subs/2009/20090324e.shtml
[2] http://biotech.da.gov.ph/Decision_docs_direct.php

2 Developments via other breeding techniques

Given the high relevance for global food security of increasing as well as securing yield under sub-optimal conditions, several approaches are being pursued. Information obtained via genotypic profiling can be the basis for marker assisted breeding. Advanced breeding techniques allow better utilization of variability, tracing of important functions (even in the absence of the possibility to observe a phenotype) and rapid introgression in performing germplasm.

As the mechanisms underlying yield development and stress tolerance gradually become unravelled, some evidence is obtained that domestication and improvements of crops that occurred in the past were based on the same strategies that are explored today via genetic engineering. Century *et al*. (2008) indicated that there is strong evidence that TFs have already played a major role in the origin of agriculture through the domestication of various crop plants. Examples include:

- Branching – nonbranching in teosinte – maize (*Teosinte branched1* (Tb1) is a member of the TCP family of TFs; will be discussed later on);
- Anti-shattering which is an important trait in the evolution of cereals, like maize, wheat etc. (*e.g.* BEL1-type homeodomain protein; and *shattering4* (SH4) that is a member of the trihelix family of plant-specific TFs and was isolated as a major QTL for shattering in a cross between *Oryza sativa* and *O. rufipogon*).

Many of the semi-dwarf but high-yielding crop varieties that were developed during the Green Revolution are defective in gibberellin biosynthesis or unresponsive to gibberellin (Peng *et al.*, 1999). The semi-dwarf varieties of wheat and rice efficiently used applied nitrogen in grain development rather than in straw elongation. The green revolution genes, wheat *Rht* and rice *sd1*, are involved in GA signalling and biosynthesis, respectively. It has been demonstrated that *Rht-B1* and *Rht-D1* (wheat) were orthologues of the *Arabidopsis* GIBBERELLIN INSENSITIVE (GAI) gene, a member of the GRAS family of TFs (Silverstone & Sun, 2000).

Yet, some authors (Richards, 1996; Tester & Bacic, 2005) indicated that traditional approaches to breeding crop plants with improved abiotic stress tolerances have so far met limited success. This is due to a number of contributing factors, including:

1) the focus has been on yield rather than on specific traits;
2) the difficulties in breeding for tolerance traits, which include complexities introduced by genotype by environment (G x E) interactions and the relatively infrequent use of simple physiological traits as measures of tolerance, have been potentially less subject to G x E interferences; and
3) desired traits can only be introduced from closely related species.

In describing efforts and challenges of improving nitrogen use efficiency in crop plants, Hirel *et al.* (2007) provide a critical overview on how understanding of the physiological and molecular controls of nitrogen assimilation under varying environmental conditions in crops has been improved through the use of combined approaches, mainly based on whole-plant physiology, quantitative genetics, and forward and reverse genetics approaches.

Cassells & Doyle (2003) stressed that the choice of plant breeding method should not be driven by technology solely but with regard to crop (whether sexually-propagated – self or cross pollinated – or clonally propagated; its use and its degree of domestication); the character(s) (whether major and/or polygenic and whether available in sexually compatible germplasm) and infrastructure (including consumer acceptance).

Mutation techniques have been used widely in efforts to breed abiotic stress tolerance and disease resistant lines with some success. The molecular effects (*i.e.* there effects on DNA) of physical and chemical mutagens are well characterized and are very similar to the spontaneous mutation arising in nature. Also, variation induced *in vitro* (somaclonal variation) has contributed to the development of abiotic and biotic stress resistant varieties in major crops (Brar & Jain, 1998).

Most cereals are moderately sensitive to a wide range of abiotic stresses, and variability in the gene pool generally appears to be relatively small and may provide few opportunities for major step changes in tolerance. Modest increases in tolerance may be introgressed into commercial lines from tolerant landraces using marker-assisted breeding approaches (Dubcovsky, 2004), facilitated by recent breakthroughs with positional cloning (*e.g.* Yan *et al*., 2003, 2004) that are likely to enable identification of extant tolerance genes within cereal germplasms.

Sinclair *et al.* (2004) report on basic physiological research at the basis of improving Water Use Efficiency (WUE) in wheat, leading to several wheat varieties being to be released from this program to growers and being marked by 'Drysdale', the first commercial variety.

There is much interest in these traits as an essential part for sustainable food production in developing countries. From the wide range of news reports, a small sample is presented here:

- In a document from FAO (2003) by the Consultative Group On International Agricultural Research, J. Bennett of the International Rice Research Institute (IRRI) presented a paper entitled Status of Breeding for Tolerance of Abiotic Stresses and Prospects for Use of Molecular Techniques (Annex I). Dr. Bennett's paper pointed out that the CGIAR Centers have a comparative advantage in these breeding activities because of their germplasm collections, their new capacity for genetic dissection of complex traits, and their ability to conduct multidisciplinary plant improvement programs in target environments. The Centers have released several high-yielding cultivars with enhanced tolerance of abiotic stresses, including Al-tolerant rice from CIAT, cold-, salt- and submergence-tolerant rice from IRRI, and drought tolerant maize from CIMMYT.
- CIMMYT and IITA work with national partners to develop drought tolerant maize in Africa[3]. As a result, more than 50 new drought tolerant varieties and hybrids have been developed and released for dissemination by private seed companies, national agencies and non-governmental organizations. African farmers now grow many of those varieties, which yield 20-50% more than others under drought, on hundreds of thousands of hectares.
- India's Central Rice Research Institute has developed a drought resistant variety of paddy for rain-fed up-plant areas[4] (the area in upper reaches) where there is little water, just about a month after a flood-resistant seed was released by the institute in Orissa[5].
- Conventional breeding programs for salinity tolerance include the development of rice, wheat and Indian mustard varieties tolerant to salt and to alkali soils by the Central Soil Salinity Research Institute in Karnal, India[6].
- NSIC Rc194 (aka Submarino 1)[7] is the IR64 rice mutant infused with submergence tolerance gene (*Sub1*), which was discovered by IRRI and the University of California-Davis from an Indian rice variety FR13A. Submarino 1 is claimed to be a non-genetically engineered rice plant that can survive, grow and develop even after 10 days of complete submergence in water at vegetative stage.
- Australian Grain Technologies launched in 2007 the drought tolerant wheat variety Gladius[8]. It is said to produce yields 20 to 30% higher than benchmark varieties in drought prone areas.

To improve the genetic potential of crops, breeders may turn to other techniques in response to the challenges they face, among which:

- limited genetic variability in the existing germplasm,
- absence of the desired trait in the existing germplasm, and
- limited and time consuming possibilities to cross and back-cross with related species.

---

[3] http://www.seedquest.com/News/releases/2009/april/25913.htm
[4] http://www.seedquest.com/News/releases/2009/june/26575.htm
[5] http://www.seedquest.com/News/releases/2009/july/26796.htm
[6] http://plantstress.com/files/salt_karnal.htm
[7] http://www.seedquest.com/news.php?type=news&id_article=9391&id_region=&id_category=&id_crop
[8] http://www.grdc.com.au/director/events/groundcover?item_id=E69EAC49ABD53B3A589E129A2C7303F5&article_id=E6AF9D32C2976DAF307B0E76C7005636

3 Developments via genetic engineering

3.1 Yield enhancement

When plants develop, many processes are co-ordinately regulated. Cells divide and differentiate in a tightly organized way, organs are formed, nutrients and energy is captured. Plant hormones are involved, signals from the environment are perceived and transferred and signalling within the plant reports on nutrient status. Yield enhancement can therefore be achieved in very diverse ways of modifying the basic functions of a plant, such as growth rate, photosynthesis, plant architecture, nutrient uptake and metabolism, ratio above-ground to below-ground biomass, transition to flowering, seed set, harvest index etc.

On the molecular level this is effected by the modification of genes and their expression. In plants protein activity is regulated transcriptionally, post-transcriptionally, post-translationally and by degradation. The result may be a modification in the synthesis of a single component or it may lead to the modification of a whole pathway.

3.1.1 Plant development

Plant development phenomena may be expressed in distinct parameters such as germination rate, growth rate, biomass development, organ size, flowering characteristics, plant architecture etc. However, different phenomena may be influenced by similar genetic elements, plant compounds and mechanisms. Therefore, this chapter is divided into subchapters, more or less according to type of genes (gene families) or belonging to a certain signalling or regulatory pathway, rather than according to the resulting trait.

3.1.1.1 Cell cycle genes

Cell division is an essential element in growth. The cycle of synthesis and division to produce two daughter cells is controlled by the so-called cell cycle genes. The rate of proliferation is dependent on the rate of completing a cycle.

Cyclin-dependent kinases (CDKs, serine/threonine protein kinases) and cyclins (CYCs) govern the plant cell cycle. Together they form heterodimers with cyclins as the regulatory subunits and CDKs the catalytic subunits. They are activated only when the heterodimers are formed. Most CDKs are constitutively expressed in cells whereas cyclins are synthesized at specific stages of the cell cycle, in response to various molecular signals (Dudits *et al*., 2007). The heterodimers phosphorylate a plethora of substrates at the key G1-to-S and G2-to-M transition points, triggering the onset of DNA replication and mitosis (Inzé & De Veylder, 2006). Particular CDK-cyclin complexes, activated at the G1-to-S transition, trigger the start of DNA replication. Different CDK-cyclin complexes are activated at the G2-to-M transition and induce mitosis leading to cell division. Each of the CDK-cyclin complexes executes its regulatory role via modulating different sets of multiple target proteins. Furthermore, the large variety of developmental and environmental signals affecting cell division all converge on the regulation of CDK activity. CDKs can therefore be seen as the central engine driving cell division (CropDesign patent WO 2000/036124).

Many cyclins exist in plants. In general D-type cyclins are thought to regulate the G1-to-S transition, A-type cyclins, the S-to-M phase control, and B-type cyclins both the G2-to-M transition and intra-M-phase control. On their turn the expression of cyclins is regulated by both intrinsic developmental signals (*e.g.* plant growth factors) and extrinsic environmental cues (Inzé & De Veylder, 2006). Plant cyclins are, as in other organisms, subject to extensive regulation by proteolysis (degradation by ubiquitination).

Besides via the formation of complexes with cyclins, the activity of CDKs is also regulated by other mechanisms like phosphorylation: CDKs are activated through phosphorylation by a CDK-activating kinase (CAK), or the CDK/CYC complex is inactivated via phosphorylation by WEE1 kinase (Inzé & De Veylder, 2006 and references therein). WEE1 is activated in response to

stress. A further level of regulation of CDK activity involves the so-called CDK inhibitors (CKIs) and in plants are designated Kip-related proteins (KRPs). KRPs are activated by cold and ABA.

The CDK/CYC complexes activate transcription factors (*e.g.* E2F family) that in turn, control the expression of many genes required for entry into and execution of S phase (DNA replication) and cell cycle progression. CDKs also phosphorylate retinoblastoma (Rb) proteins that are repressors of cell cycle transcription factors of the E2F family.

Slowing down the cell cycle decreases cell production and thus the number of cells in an organ. But the smaller number of cells is typically at least partly compensated by having larger cells (Beemster *et al*., 2005; Barrôcco *et al*., 2006).

Modifying the expression of proteins that are involved in cell cycle regulation with the aim of increasing a plant's yield is subject to different patents by the company CropDesign.
Some examples:

- Using expression of a CDC2 protein for accelerating and increasing the production of biomass (*cdc* was used to name the first cell division cycle genes discovered in yeast; today *cdk* is preferred) (WO 2000/052172);
- Increasing seed yield using a B-cyclin (CYCMs2) gene driven by an inducible or seed specific promoter (WO 2000/052169);
- Increasing cell division rates and growth rates using CDKs and cyclins or other cell cycle interacting proteins (WO 2000/056905);
- Dominant negative mutation of CDC2a (CDC2a-DN) to alter plant architecture in *e.g.* tomato (WO 2001/031041);
- Over-expressing of a CYCD to increase overall growth rate (WO 2001/096579);
- Enhanced growth rate, stress resistance and seedling survival, more cells, more tillers, more panicles by tissue specific over-expressing a member of the E2F transcription factor family (WO 2003/025185);
- Modifying growth rate, yield, senescence, flowering and photosynthesis by modulating expression of a plant cyclin dependent kinase-like gene (WO 2003/027299);
- Modifying growth characteristics by reducing or substantially eliminating retinoblastoma 1 (Rb1) in a plant (WO 2004/016775). Rb proteins interact with the E2F/DP complex to repress transcription of E2F-regulated genes;
- Accelerating the rate of development, increasing size and number of organs and promotion of early flowering, by increased or decreased expression of a gene encoding a CDC27A (WO 2004/029257);
- Constitutively over-expressing a B-type *CDK* gene to increase seed yield (WO 2005/024029);
- Over-expressing of a CYCA protein in seeds to enhance seed yield (WO 2005/061702);
- Over-expressing of a CYCD3 protein in shoots to increase seed yield (WO 2005/085452);
- Constitutively over-expressing of a CDKD to enhance seed yield (WO 2005/083094);
- Over-expressing a mutant rice CDKA kinase in shoots for an increased seed yield (WO 2006/058897);
- Over-expressing of a CYCD3 in the endosperm to increase seed yield (WO 2006/100112); and
- Increasing activity of an E2F Dimerization Partner (DP)-encoding gene (DP protein) in shoot tissue to increase growth rate, biomass and seed yield (WO 2005/117568). DP factors act together with E2F factors to form a heterodimer, capable of initiating transcription of S-phase specific genes.

Examples of patent applications by other companies include:

- ArborGen, US 7,371,927: modulating growth and biomass of trees or shrubs using a promoter that is active in wood-forming tissues and a CYCD polynucleotide sequence in combination with a sequence encoding an enzyme involved the production of cell wall materials, such as lignin and high crystalline cellulose in wood;
- ArborGen, US 7,598,084: modifications of plant traits using CYCA;
- BASF Plant Sciences, WO 2007/141189: shoot-specific over-expression of an A-type CDK to increase growth rate and seed yield; and

- Targeted Growth, WO 2007/016319: a mutant KRP protein to inhibit the wild-type KRP protein so as to increase plant vigour, germination rate, root mass, seed size of the transgenic plant depending on the developmental and space specificity of the corresponding wild-type KRP. The wild-type CKI biological activity is inhibited, leading to accelerated progression through the cell cycle and increased cell proliferation.

3.1.1.2 Genes connected to plant hormones

Growth within determinate plant organs (*i.e.* leaves and flowers) can be considered to consist of two phases. Initially cellular growth is coupled with cell division leading to an overall increase in cell number within the developing organ. Subsequently, cell division ceases and further growth of the organ results from cell expansion.

By way of example the plant hormones auxin and brassinosteroids are important regulators of plant growth, stimulating both cell division and cell elongation (Krizek, 2009). Figure 2 exemplifies the complex interactions of hormones and regulators.

On top of these mechanisms the phenomenon of imprinting maternal and paternal alleles plays a role with regard to seed size (endosperm) in flowering plants. It has been proposed that paternal genes promote seed growth, whereas maternal genes rather reduce growth; or, conversely, that in the maternal genome growth promoting factors are inactivated.

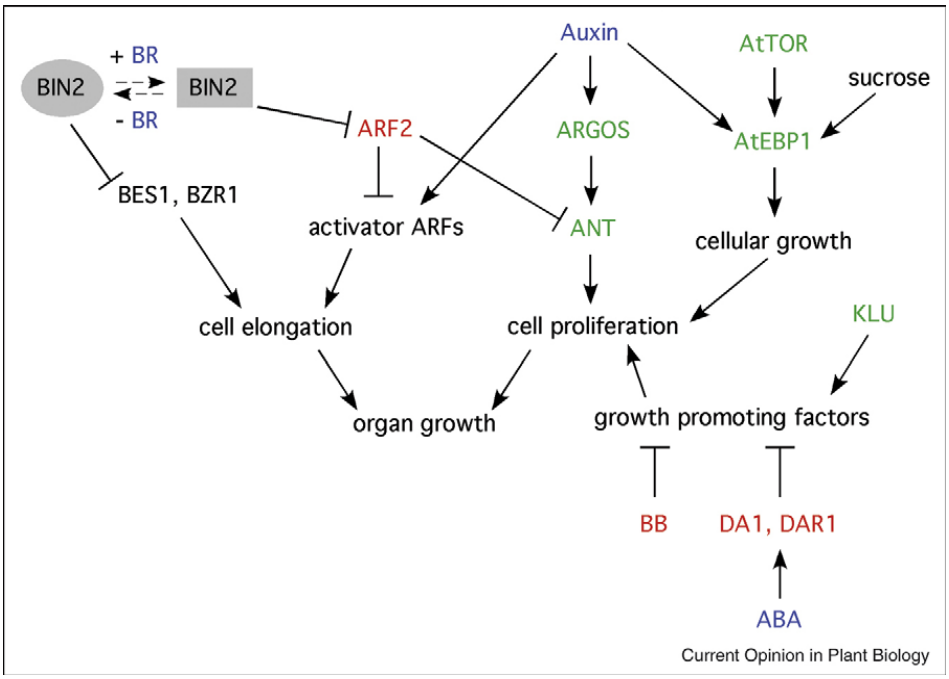

Fig.2 Pathways controlling final organ size. Plant hormones are shown in blue, proteins promoting growth are shown in green and proteins restricting growth are shown in red. Auxin promotes growth through ARGOS and ANT while ARF2 negatively regulates ANT expression. ARF2 is negatively regulated by BIN2 allowing integration of auxin-signalling and BR-signalling pathways. The target specificity of BIN2 may be regulated by BRs. KLU promotes growth through the production of a novel growth-promoting signal while BB, DA1, and DAR1 restrict growth through negative regulation of unidentified growth-promoting factors. AtTOR and AtEBP1 likely regulate macromolecular synthesis and cellular growth to promote organ growth. AtEBP1 expression is induced by sucrose and AtEBP protein is stabilized by auxin. (taken from Krizek, 2009; copyright 2009 Elsevier, with permission)

3.1.1.2.1 Gibberellins

Gibberellins (GAs) are plant hormones involved in growth and various developmental processes, including stem elongation, germination, dormancy, transition from the vegetative state to flowering, sex expression, and leaf and fruit senescence.

Over hundred gibberellins (GAs) are known in plants. Only a few are thought to be bioactive (Yamaguchi, 2008), so many exist as precursors. The concentration of the different GAs is the result of synthesis and deactivation rates. Both GA biosynthesis and deactivation pathways are tightly regulated by developmental, hormonal, and environmental signals, consistent with the role of GAs as key growth regulators (Yamaguchi, 2008). The early steps in biosynthesis are encoded by 1 or 2 genes, the later steps by multi-gene families. The members in each of the families are differentially regulated and their roles overlap partially. Influencing the levels of GAs and therefore plant development, might be done by modifying the expression of genes coding for enzymes involved in the synthesis or degradation or their regulators.

Over-expression of genes for biosynthesis often results in increased levels of bioactive GAs, followed by accelerated plant development (Pimenta Lange & Lange, 2006). GA 20-oxidases catalyze rate-limiting steps of the pathway and in that way they are capable of controlling plant development. Over-expression of GA 20-oxidase 1 in *Arabidopsis* gives rise to seedlings with longer hypocotyls and petioles, larger rosette leaves, accelerated flowering and bolting compared to controls in both long and short days and are 25% taller at maturity (Coles *et al*., 1999). Plants grow higher and faster. On the contrary, repression of some GA 20-oxidases by antisense approaches in *Arabidopsis* plants results in shorter stems (Coles *et al*., 1999).

Over-expression of GA 2-oxidases offers a direct way to decrease GA levels and, by this, to retard plant development. This has been observed for some plant species, including poplar, rice, and tobacco (Busov *et al*., 2003; Sakamoto *et al*., 2003; Biemelt *et al*., 2004). These over-expressors are not capable of germinating or flowering.

Dwarf plants may produce higher yields by diversion of assimilates from vegetative to reproductive organs. They will present a more favourable harvest index. However, a straightforward constitutive expression is often not the way to go. To achieve the intended effect tissue or organ specific promoters are often used as in the transgenic dwarf rice (Sakamoto *et al*., 2003). Constitutive expression of the rice GA 2-oxidase 1 in rice led to severe dwarfism and failure to set grain. However, over-expression of the same GA 2-oxidase in rice under the control of a rice GA 3-oxidase 2 promoter (*d18* mutant) resulted in semi-dwarf phenotype with normal flower and grain development. This promoter is not active during flower and grain development (Sakamoto *et al*., 2003).

Field trial permits have been submitted for poplar transformed with GA 20 oxidase (US notifications for GM poplar: 05-130-01N and 06-250-01R, poplar gene; 05-341-04N and 07-128-101R, cotton gene) to induce dwarfism, probably by anti-sense technology, or to enhance growth. As tested in poplar this approach may also be useful for fruit trees. High density planting systems in fruit production require dwarf trees for limiting plant height and thus allowing easy management as well as achieving high production efficiency and precocious and profuse flowering (Busov *et al*., 2003). In case of wood production stands may have higher biomass productivity due to reduced investment in root mass by shorter trees, lower moisture stress and lower respiratory surface area of the bole (Busov *et al*., 2003).

In transgenic Carrizo citrange (*Citrus sinensis x Poncirus trifoliata*), over-expressing *CcGA 20-oxi1* in antisense induces a decrease of the accumulation of GA_1 in growing shoots of the transgenic plants. Consequently, antisense plants are semi-dwarf. The ultimate goal is to produce rootstocks that modulate the growth of non-transgenic *Clemenules Clementine* that are grafted on them. Field trials were applied for in Spain (applications B/ES/06/43 and B/ES/08/03).

The Central Potato Research Institute in India received a permit in 2009 to select potato events transformed with a GA 20-1 gene in the field.

In sugarcane the barley *HvGA20ox-1* and *HvGA20ox-2* genes were used to obtain taller plants (Australian applications DIR070/2006 and DIR095/2009). The promoters that are driving these genes are not disclosed apart from the maize ubiquitin promoter. In the first trial a 50% increase was noticed using the Ubi promoter. In the greenhouse the plants turned out to produce more stalks more rapidly (1-2 months earlier). In the field they produced less stalks. In the same applications also a runner bean PcGA 2-oxidase with the same promoter was introduced in sugarcane to make shorter plants (approximately 35% shorter). These plants grew more slowly. Stalk number was increased with 30% in the first trial. In DIR070/2006 also the use of HvGA3ox-2 is mentioned. Other field trials with GA 2-oxidase are reported for poplar (US applications: 07-080-104N, 06-250-01R and 06-137-117N *Phaselous coccineus* and poplar genes; 06-069-05N *Phaselous coccineus* gene) and Bahia grass (US applications: 07-197-127R and 05-364-01R, *Arabidopsis* gene). Bahia grass is an amenity grass in parts of the USA and the aim here is to reduce mowing requirements.

Dwarfing by inserting GA 2-oxidase gene constructs may also be useful to prevent lodging in cereals, to prevent lodging in oilseed rape, to improve the canopy structure, and to prevent pre-harvest sprouting in cereals (US patent 7,262,340). Therefore this trait can function as a way to preserve harvest.

The levels of bioactive GAs are maintained via feedback and feedforward regulation of GA metabolism establishing homeostasis:
- Although the mechanism is not yet understood, GA receptors certainly play a role like in the case of the Gibberellin Insensitive Dwarf1 (GID1).
- Homeobox genes like *KNOTTED1-like* and *SHOOTMERISTEMLESS*, MADS-box genes like *AGAMOUS* and *AGL15*, and leucine-zipper genes like *REPRESSION OF SHOOT GROWTH* (*RSG*) control levels of GAs acting on their biosynthesis or deactivation genes (Yamaguchi, 2008 and references therein).
- Auxins like indole-3-acetic acid (IAA) up-regulate GA3ox1 and down-regulate GA2ox1.

In GA signal transduction the DELLA proteins play a prominent role. They are negative regulators repressing GA-induced gene transcription (Salas Fernandez *et al*., 2009). REPRESSOR-OF-GA1-3 (RGA) and GIBBERELLIC ACID INSENSITIVE (GAI) are two examples of DELLA proteins in *Arabidopsis*. SLENDER RICE1 (SLR1) is a rice DELLA protein. As soon as GA binds to GID1, this protein binds to SLR1 leading to ubiquitination and proteolysis of SLR1, mediated by GID2. The repressor is then removed and GA-induced gene transcription can start (Salas Fernandez *et al*., 2009; Schwechheimer & Willige, 2009 and references therein).

Examples of the use of regulators in GA biosynthesis are
- KNOTTED-1 to alter development in maize (US notifications: 95-100-03N and 94-161-01N, maize gene);
- RGL1 Negative Regulator of Gibberellin Responses in poplar (US notifications: 07-080-104N and 06-137-117N, *Arabidopsis* gene);
- Repressor of gibberellin response in rapeseed to reduce plant height (US notifications 05-245-04N and 05-245-05N, *Arabidopsis* gene);
- The soluble GA receptor GID1 in oilseed rape (US notifications: 07-234-103N; 07-234-102N and 07-234-101N, *Arabidopsis* gene) improves lodging resistance by reducing the stature of the plants; and
- A sequence encoding a mutant of the transcription factor GAI (gibberellic acid insensitive) is used to dwarf oilseed rape increasing lodging resistance (US notifications: 06-219-104N, 06-226-104N and 06-226-103N, *Arabidopsis* gene). The wildtype *GAI* gene encodes a repressor of gibberellin responses which is derepressed by gibberellin itself. The mutant gene, *gai*, has lost the ability to be derepressed by gibberellin and thus continually suppresses gibberellin responses causing a dwarf phenotype. Again, in poplar this gene is used to produce dwarf trees (US notification 05-158-02N, *Arabidopsis* gene).

Gibberellins also influence the transition from the vegetative to the generative phase. The shift from meristem identity to organ differentiation is regulated via KNOX proteins. KNOTTED1-like homeobox proteins suppress GA biosynthesis (*GA20ox* expression) and activate cytokinin (CK)

synthesis in the corpus of the shoot apical meristem (SAM). Without KNOX expression, suppression of *GA20ox* expression is alleviated in leaf primordia. The expression of GA deactivation genes (*GA2ox*) at the base of the meristem induced by SHOOTMERISTEMLESS may assist the establishment of a low-GA regime in the SAM. The homeotic gene *AGAMOUS* (*AG*) is expressed after flower induction, terminates meristem activity, and promotes development of floral organs. It does so by elevating expression of *AtGA3ox1*, which may cause an increase in GA levels in the floral meristem and promote the shift from meristem identity to differentiation (Yamaguchi, 2008).

Bioactive GAs function as key mediators between the perception of environmental signals and the resulting growth responses. Seed germination is a typical example of a response to light. In seeds phytochromes (PHYA and PHYB) stimulate germination elevating expression of AtGA3ox1 and AtGA3ox2, whereas AtGA2ox2 expression is suppressed, resulting in an increase of active GA_4. In the absence of active phytochrome, PHYTOCHROME-INTERACTING FACTOR 3–LIKE 5 (PIL5) suppresses *GA3ox* expression and activates *GA2ox* expression. Once phytochrome is activated by red (R) light (Pfr), the PIL5 protein is degraded. This allows for the up-regulation of *GA3ox* genes and causes the down-regulation of *GA2ox* expression. As a consequence, GA_4 levels are elevated and germination is stimulated. PIL5 acts indirectly via binding to the promoters of the DELLA proteins RGA and GAI (Yamaguchi, 2008). DELLA proteins have also been shown to function as repressors of the PHYTOCHROME INTERACTING FACTOR3 (PIF3) and PIF4 transcriptional activators in the context of light-regulated seedling development (Schwechheimer & Willige, 2009 and references therein).

YABBY1 (OsYAB1) may be a mediator of GA homeostasis downstream of the DELLA protein in rice (Yamaguchi, 2008). YABBY proteins are reported to promote abaxial cell fate in lateral organs. Patent application WO 2008/059048 reports that the YABBY protein CRABS CLAW, that promotes nectary development and carpel fusion, also enhances abiotic stress tolerance when constitutively expressed.

3.1.1.2.2 Brassinosteroids

Brassinosteroids (BRs) promote cell elongation, control tiller number, leaf size, and leaf angle, play an important role in controlling seed size and weight, and influence photosynthetic CO_2 assimilation (Wu *et al*., 2008 and references therein). Brassinolide (BL), the most active BR, is synthesized involving DWARF7 (DWF7), DWF1, FAD dependant oxidase, DEETIOLATED2 (DET2) steroid 5a-reductase and several cytochrome p450 monooxygenases, such as DWF4, CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM (CPD) and DWF (Salas Fernandez *et al*., 2009 and references therein). Examples of enzymes involved in the biosynthesis of BRs in rice include OsDWARF2, OsDWARF11 and BR-deficient dwarf-1 (BRD1) and BRD2. DWF4 and CPD, are down-regulated in the presence of BRs.

Wu and colleagues (2008) introduced sterol C-22 hydroxylases that are rate limiting in BR biosynthesis in rice, provided with a promoter active in stems, leaves, and roots. A dramatic increase in seed yield was the result, both in greenhouse experiments and field trials in China. This was due to increased tillering resulting in more seeds per plant, but also to heavier seeds. The latter might be the result of better CO_2 assimilation followed by enhanced sucrose transport to the developing kernels.

DET2 was used in poplar (US application 07-128-101R, cotton gene), possibly in combination with a GA20ox, to increase plant height and/or alter the flowering time. Often when plants grow taller, flowering is delayed.

Transgenic rice constitutively over-expressing the rice DWF1 orthologue have been produced, and typically display increased plant height, increased internode length, and increased number of spikelets per panicle (Hong *et al*., 2005). DWF1 is also mentioned in patent application WO 2008/092910 claiming that, when over-expressed in rice using the rice *GOS2* promoter, the plants have an increased seed yield.

BR biosynthesis is probably feedback-inhibited by the BR signalling pathway. BRs are sensed by a membrane-bound receptor called BRASSINOSTEROID INSENSITIVE 1 (BRI1). BRI1 sense and antisense was trialled in cotton (US notification 08-073-110N, *Arabidopsis* gene) to increase yield. It was probably the intention to dwarf the plants and as a consequence increase the harvest index.

3.1.1.2.3 Auxins

Hormones play a critical role in regulating branching (McSteen & Leyser, 2005). Auxin is required for axillary meristem initiation during both vegetative and inflorescence development.

In application DIR070/2006 genes were included relating to auxin effects. OsMAX3, OsMAX4-1 and OsMAX4-2 from rice and SoMAX3 from sugarcane (more axillary growth) are proteins that affect auxin transport in the plant (McSteen & Leyser, 2005). These proteins are divergent carotenoid cleaving dioxygenases, suggesting a novel regulatory pathway involving carotenoid-derived signalling molecules. In the sugarcane lines complete sequences were inserted to enhance expression as well as partial sequences intended to suppress expression.

Trials on maize by Cold Spring Harbor Laboratory (US notifications 07-065-125N; 08-142-108N; 09-117-107N) use the maize PINFORMED1 (PIN1) auxin efflux facilitator to decrease branching in maize. Localized on the plasma membrane, PIN1 actively transports auxin out of cells. In this way the protein establishes an auxin gradient in order to start primordia (Gallavotti *et al*., 2008).

Several genes that influence tillering in monocots have been identified, such as *MONOCULM1*, *TEOSINTE BRANCHED1* (*TB1*) and *HIGH-TILLERING DWARF1* (*HTD1*) (Wu *et al*., 2008 and references therein). Auxin affects the expression of *HTD1*.

Inflorescence morphology is a major yield factor in many crops, and is determined by the activities of shoot meristems. Development in axillary meristems is initiated involving genes like *REVOLUTA* (*REV*) and *LATERAL SUPPRESSOR* (*LAS*). *REV* encodes a homeodomain/leucine zipper transcription factor and is expressed in very early axillary meristems. Examples of patents involving *REV* are:

- Targeted Growth, WO 2007/079353 increasing the seed size and/or seed number in plants using a *REV* gene driven by an early phase-specific embryo promoter; and
- Pioneer HI-Bred, WO 2004/063379, modulating meristem development using a maize *REVOLUTA* gene: a *REVOLUTA* sequence having dominant negative activity will decrease the activity of the endogenous REVOLUTA polypeptide so as to enhance the growth of meristem structures by regulating cell division in the meristem.

The maize teosinte branched (*TB1*) locus is another well-characterized regulator of axillary meristem growth, that acts downstream of LAS. The basic helix-loop-helix (bHLH) proteins are a superfamily of transcription factors that bind as dimers to specific DNA target sites. The plant bHLH proteins have been reported to function in anthocyanin biosynthesis, phytochrome signalling, globulin expression, fruit dehiscence, carpel and epidermal development (Buck and Atchley, 2003). The maize *TB1* gene encodes a protein with a bHLH domain and belongs to the TCP transcription factor family (named after its first characterized members: TB1, CYC and PCFs).

Transgenic wheat containing a gene construct with the maize ubiquitin promoter and the maize *TB1* coding sequence produced less tillers (Lewis *et al*., 2008). In Australia GM sugarcane lines will be trialled on 6 locations for 6 years (DIR095/2009). To reduce tillering the *OsTB1* gene sequence from rice or the *ShTB1* gene from sugarcane had been inserted. An RNAi construct for ShTB1 induces silencing of the endogenous gene and therefore increase the numbers of tillers. In a previous trial (DIR070/2006) similar gene constructs were assessed. *OsTB1* lines (Ubi promoter) showed a 25% decrease in stalk number. The *ShTB1* RNAi construct increased the number with approximately 20% (Ubi promoter). In the US field trials were notified for maize transformed with maize *TB1* with or without other genes that might influence inflorescence architecture (US notifications 05-112-01N and 07-080-106N).

GM maize modified with teosinte glume architecture 1 gene (*TGA1*) isolated from maize is claimed to show what is described in general terms as altered flowering (US notifications 05-112-01N, 06-018-12N and 07-080-106N). TGA1 controls an important step in the evolution of fruitcase structure, and was a major element in maize domestication.

Protein ubiquitination is a post-translational regulatory process essential for plant growth and interaction with the environment. SINA (seven in absentia) proteins are E3 ligases that determine the specificity by selecting the target proteins for ubiquitination. The *Arabidopsis* SINAT5 attenuates the auxin-induced lateral root formation. Ectopic expression of a dominant-negative mutant of the SINAT5 (SINAT5DN) causes more lateral roots than in wild-type plants (Xie *et al*., 2002). SINAT5 and SINAT5DN were both trialled in peanut (US notification 09-103-109N, *Arabidopsis* gene) and cotton (US notification 09-093-113N, *Arabidopsis* gene).

3.1.1.2.4 Cytokinins

Cytokinins are involved in cell division and influence seed germination, shoot/root balance, transduction of nutritional signals, leaf expansion, reproductive development, and delay of senescence (Sakakibara, 2006). Cytokinin biosynthesis and homeostasis are finely controlled by internal and external factors such as other phytohormones and inorganic nitrogen sources. They act at various sites in a plant.

Plant adenosine phosphate-isopentenyltransferases (IPTs) are of the first enzymes in the cytokinin biosynthesis pathways starting from adenosine 5′-phosphates leading to zeatins and N^6-(Δ^2-isopentenyl)-adenine. The different genes show tissue- and organ-specific expression patterns. Plastids are the major subcellular compartment for this initial step. Cytokinins are a pivotal signalling substance communicating the nitrogen nutrient status from root to shoot via the xylem vessels. This is established through nitrogen-dependent IPT activity and regulation.

Cytokinins can be catabolized by cytokinin oxidase (CKX) to adenine or adenosine (Sakakibara, 2006). Genes for CKXs are regulated by cytokinins and ABA. In rice the OsCKX2 is identified as being the QTL that increases grain productivity (Ashikari *et al*., 2005). OsCKX2 degrades cytokinin that controls the formation of rice inflorescence meristems. Reduced expression of OsCKX2 causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, resulting in enhanced grain yield (Ashikari *et al*., 2005).

In the US maize notifications 07-065-125N, 08-142-108N and 09-117-107N by Cold Spring Harbor Laboratory, also CKX1 and CKX2 were inserted together with isopentenytransferase 2 (IPT2), all isolated from maize, to alter inflorescence development and to increase seed number. Also included were genes for RESPONSE REGULATOR1 (RR1) and 7 (RR7). These proteins are negative regulators of cytokinin signalling (Müller & Sheen, 2007). Histidine kinases (HKs) and histidine phosphotransfer proteins (HPTs) play a role in cytokinin signal transduction (Müller & Sheen, 2007). The contribution of these genes was tested in the same maize fields.

Yet another gene is included in these trial applications, *ABERRANT PHYLLOTAXY1 (ABPHYL1* or *ABPH1*). *ABPH1* is a cytokinin-inducible response regulator that is involved in establishing the arrangement of leaves and flowers in the shoot apical meristem (SAM), phyllotaxy (Lee *et al*., 2009). ABPH1 reveals to be a negative regulator of SAM size and a positive regulator of PIN1 expression. In these trials also PIN1, RAMOSA3 (see below), ALPHA TUBULIN, involved in cytoskeleton assembly, and FASCIATED EAR2 (see below) were studied.

Meristem size in *Arabidopsis* is regulated by the CLAVATA (CLV) proteins, including a transmembrane leucine-rich repeat kinase (CLV1). CLV mutants have larger shoot meristems and increased floral organ number, often fasciated. In maize *fasciated ear2* (*fea2*) mutants develop larger meristems during inflorescence and floral shoot development, and ear inflorescence meristems show severe fasciation, suggesting that FEA2 normally acts to limit the growth of these meristems. The gene encodes a membrane localized leucine-rich repeat receptor-like protein (Taguchi-Shiobara *et al*., 2001)), homologous to CLV2 in *Arabidopsis*. *Fea2* ears have up to twice the normal number of rows of seed. In US notifications 06-087-12N and

05-117-10N for maize trials this gene was tested for crop improvement, next to the trials mentioned above.

The use of a CLV3-like maize gene is patented by Pioneer (US patent 7,179,963). The maize *clv3-like* mutants produce mildly fasciated ears. Dominant-negative CLV3-like polypeptides interfere with the function of the normal, endogenous CLV3-like protein, thereby blocking it.

Biogemma filed a patent (WO/2007/093623) on the use of *ZmTCRR-1* gene to increase kernel size and seed weight in maize using an endosperm specific promoter. ZmTCRR-1 is a response regulator that acts as a signal transduction protein to influence cellularization, mitosis and differentiation of grain tissues. In wild type plants it is expressed in the basal endosperm transfer layer. A plausible role for ZmTCRR-1 is the transmission or modulation of the cytokinin signal in the endosperm.

3.1.1.3 Flowering genes

The timing of flowering is a major determinant of seed yield and varies depending on the local environment and crop production system (Jung & Müller, 2009). In cereals, flowering should be as early as possible to extend the seed filling phase, to avoid harsh environmental conditions which endanger seed production or harvest (*e.g.* drought, heat, frost) or to escape pathogen attack. By contrast, delayed flowering might be desirable to realize high yields in biomass for energy production.

The determination of a plant to flower is influenced by four factors affecting specific pathways: photoperiod, vernalization, autonomous and gibberellic acid (GA) pathways. Communication between the pathways occurs via floral pathway integrators (FPIs) that act to integrate flowering signals and serve as gates to activate the flowering pathway (Blázquez *et al*., 2000).

- In *Arabidopsis* the photoperiod pathway acts predominantly through *CONSTANS* (*CO*) to activate the small signalling molecule FLOWERING LOCUS T (FT) (see Figure 3). The circadian-regulated transcription factor CO is a zinc-finger protein and is one of the most conserved flowering responses among distantly related plants;
- Gibberellins promote flowering through the activation of genes encoding the floral integrators SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS 1 (SOC1), LEAFY (LFY), and FT in the inflorescence and floral meristems, and in leaves, respectively (Mutasa-Göttgens & Hedden, 2009);
- In the meristem, vernalization promotes flowering through the epigenetic repression of the floral repressor *FLOWERING LOCUS C* (*FLC*) via repressive histone modifications (Michaels, 2009). FLC blocks *FT* transcription. In the absence of FLC this allows for the induction of floral integrators by *CONSTANS*; and
- In the 'autonomous' floral-promotion pathway, a group of genes act to constitutively repress *FLC* (Michaels, 2009).

Several examples of transgenic plants having an early or delayed flowering phenotype using these and other genes are listed in Jung & Müller (2009).

- The *FVE* gene is active in the autonomous pathway. The *Arabidopsis* loss-of function mutant has a late flowering phenotype. It acts upstream of LFY and FT. FVE encodes a protein having 6 copies of a WD40 domain motif, that are believed to be involved generally as a component of transcription regulators controlling a variety of cellular processes. Inhibiting FVE expression leads to increased biomass production (Patent application WO 2009/040665);
- In field trial applications 06-076-02N, 07-069-101N, 07-315-101R and 08-065-112N *CONSTANS* isolated from *Populus deltoides* is mentioned to modulate flowering in poplar.
- As already described GA20ox from cotton was introduced in poplar (US application 07-128-101R) to modify flowering; and
- Tobacco has been transformed with the *FLC* gene to delay flowering and increase biomass (Salehi *et al*., 2005). Edenspace pursuit the same in tobacco as a model crop for producing high yielding energy crops with increased cellulose hydrolysis (US application 06-088-01R, *Arabidopsis* gene).

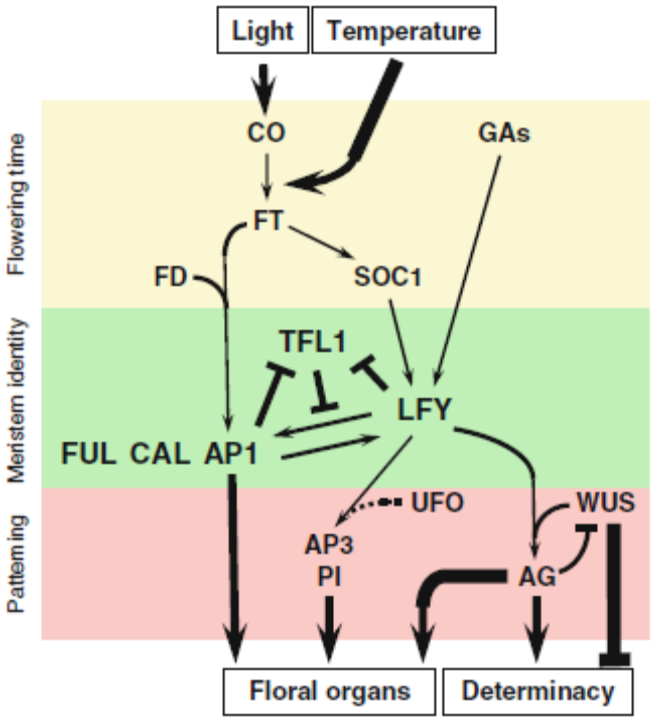

Fig.3 Schematic representation of the interactions involved in the specification of floral meristems (from Blázquez *et al.*, 2006; copyright 2006 Springer, with permission)
Abbreviations: AG: AGAMOUS; AP1: APETALA1; AP3: APETALA3; CAL: CAULIFLOWER; CO: CONSTANS; FD: FLOWERING LOCUS D; FT: FLOWERING LOCUS T; FUL: FRUITFULL; GAs: Gibberellins; LFY: LEAFY; PI: PISTILLATA; SOC1: SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS 1; TFL1: TERMINAL FLOWER1; UFO: UNUSUAL FLORAL ORGANS; WUS: WUSCHEL

After floral transition meristem identity genes and still later organ identity genes become active, many of them are so-called MADS box genes. MADS-box genes constitute a large gene family of eukaryotic transcriptional regulators involved in diverse aspects development. They encode a strongly conserved MADS domain responsible for DNA binding to specific boxes in the regulatory region of their target genes. The gene family can be divided into two main lineages, type I and type II. Type II genes are also named MIKC-type proteins.

The *Arabidopsis* APETALA1 (AP1) MADS-box protein is required for the transition from inflorescence meristems to floral meristems. Mutants losing the AP1 function together with CAULIFLOWER mutations show a reiterating process of inflorescence meristem building and only in a late stage floral identity is acquired. Ectopic constitutive expression induces early flowering (Blázquez *et al.*, 2006 and references therein).

AP1 was introduced in *Citrus sinensis* (B/ES/08/05) to induce early flowering in the transgenic plants and their progeny, thus generating rapid cycling trees. The intended field trial with transgenic oranges over-expressing the *A. thaliana* AP1 gene aimed to investigate the possible modification of tree growth due to profuse flowering and the possible modification of flower and fruit development, next to fruit quality characteristics. Constitutive expression of the meristem-identity genes for LEAFY or AP1 reduces generation time to 1 year in *Citrus* (Peña *et al*., 2001).

OsMADS15, the rice orthologue for the *Arabidopsis* AP1, was postulated to function in a complex with other proteins to control organ formation. Patent application WO 2007/113237 describes the transformation of rice with a GOS2-OsMADS15 construct to enhance root biomass or, if using co-suppression, antisense or RNAi or other technology to reduce expression of the endogenous gene, to increase seed yield and/or biomass.

OsMADS18 resembles most the maize ZMM28. Constitutive over-expression of OsMADS18 in rice leads to dwarfed plants and earlier flowering as a consequence of accelerated plant maturation. Expression from the embryo globular stage up to the young seedling stage (early shoot apical meristem promoter) improves the seed weight (WO 2006/056590).

Also ZMM28 that is expressed predominantly in ears was proposed for use in transgenics enhancing yield through controlling the number of spikelets per ear, final kernel number and kernel size (WO 2008/148872). This transcription factor is the maize counterpart of the *Arabidopsis* AP1. AP1 from *Arabidopsis* or the poplar homologue has been inserted in poplar and notified for field trials in the USA (US applications 06-076-01N, 06-250-01R, 06-263-107N, 06-270-113N, 06-282-101N, 06-282-102N, 06-282-103N, 07-069-101N, 07-268-101N, 07-268-102N, 07-268-103N, 07-315-101R, 08-065-112N). However, it is not clear whether the purpose is related to wood yield increase or the intention to prevent gene dispersal by delaying flowering or producing sterile flowers.

The role of MADS-box organ identity genes in flower development is explained in the ABC model (Coen & Meyerowitz, 1991) that was later extended to the ABCDE model. The A function is required for sepal specification, ABE together for petals, BCE for stamens, CE for carpels and D, together with C and E for the determination of ovules.

- APETALA2 (AP2) is an A function type protein of the plant specific AP2 transcription factor family. When the poplar counterpart is introduced in poplar, claims of bigger leaves have been made (US notification 07-086-105N) thereby increasing the leaf area index (more photosynthesis). In tomato using the *Arabidopsis* gene (US notification 07-078-101N) yield was increased.
- The maize MADS box transcription factor SILKY1 is expressed predominantly in tassels and ears. It has a B function in the ABC model corresponding to AP3 in *Arabidopsis* (Ambrose *et al*., 2000). In transgenic plants it may enhance yield through controlling the number of spikelets per ear, the final kernel number and kernel size (WO 2008/148896).
- An example of a C function floral organ identity gene is the *Arabidopsis* gene encoding AGAMOUS (AG).

These floral identity genes are not only expressed in the generative phase, they also have a regulatory role in vegetative organ identity. *Glossy15* homeotic regulatory gene is an *APETALA2-like* gene from maize that regulates leaf epidermal cell identity. This transcription factor is required in maize for wax formation on juvenile leaves (Moose & Sisco, 1996). Further studies have shown that the main function of Glossy15 is to slow down shoot maturation. Having a prolonged juvenile vegetative phase results in higher biomass, making a good silage maize or energy crop (Moose, 2009). Field trials were conducted during several years (US notifications 09-058-109N, 08-089-101N, 07-090-101N, 06-086-11N and 05-049-26N),

On the other hand late flowering is regarded as a major factor limiting breeding progress in tree species.

Oregon State University in its field trial application for poplar lists genes that encode AGAMOUS, APETALA1 and RGL1 from *Arabidopsis* gene, PSVP, PAGL20 (MADS 5 homologue), PCEN-L (TFL1 homologue), PFPFL1, PFPFL2, PFT, PMFT and PAGL24 (MADS 9 homologue) from *Populus trichocarpa* to modify flowering (delaying / preventing or promoting) by over-expressing these genes or using RNAi technology (US notifications 06-263-107N, 06-270-113N, 06-282-102N, 06-282-103N, 06-283-101N, 07-268-101N, 07-268-102N and 07-268-103N), or in combination with GA 2-oxidases and GA 20-oxidases (US application 06-250-01R) to alter the plant's growth. Preventing or delaying the onset of flowering might be a strategy for biocontainment of transgenics in general and GM poplar in particular; it also results in increased biomass (Strauss, 2006). Indeed, tall plants correlate with late flowering (Salas Fernandez *et al*., 2009).

Oregon State University also conducted field trials with sweetgum (*Liquidambar*) transformed with a dominant negative mutant AG (AG-m3) and a RNAi construct for LSAG / LAG

(*Liquidambar* homoloques of AG) to build sterile flowers (Strauss, 2005; US notifications 05-305-03N, 05-355-01N, 06-286-102N, 06-286-103N, 07-232-102R, 07-277-102N).

Phytochromes are red and far-red light photoreceptors and are encoded by five different genes (*PHYA* to *PHYE*) in *Arabidopsis* (Bae & Choi, 2008). They are responsible for regulating various red light responses, including seed germination, seedling photomorphogenesis, shade avoidance, flowering, and many other adaptive responses. The biologically inactive form is called Pr. Upon absorption of red (R) light the molecule structurally changes and becomes active: the Pfr conformation. Far-red (FR) light induces the reverse reaction. The various phytochromes play overlapping but distinct roles. In *Arabidopsis*, both PHYA and PHYB promote seed germination and de-etiolation in response to FR and R light, respectively. PHYB inhibits shade avoidance responses under a high ratio of R:FR light, whereas PHYA inhibits excessive shade avoidance responses under a low ratio of R:FR light; PHYA promotes flowering, whereas PHYB delays flowering (Bae & Choi, 2008 and references therein). PHYA and PHYB regulate the circadian activity of CO. PHYA stabilizes CO, PHYB promotes the degradation of CO. This means that if expressed from a constitutive promoter, CO protein accumulates under white, blue, or far-red light, but is degraded in red light or darkness. GM plants having only a *CO* construct might not be enough to change flowering time.

The Mississippi State University mentioned in its field trial applications with poplar the use of *CONSTANS1, CONSTANS2, PHYA, PHYB, FLT2, AP1, GIBBERELLIN INSENSITIVE* from *Populus deltoides* to alter flowering time and floral development (US notifications 08-065-112N, 07-315-101R, 07-069-101N, 06-076-02N, 06-076-01N).

Plants try to catch as much light as possible. Growing in a dense crop stand they tend to grow higher than their neighbours, a phenomenon called shade avoidance response. Plant resources are essentially redirected from leaves and storage organs into increased extension growth and decreased branching. Other, less immediate, responses include accelerated flowering and early, but reduced production of seeds. Repressing this response might induce higher yields. This would only be effective in a monoculture of shade avoidance repressed plants.

As plant canopy grows and fills up space, a reduction in the ratio of R/FR light occurs because FR light is filtered through or reflected by vegetation. Both PHYA and PHYB sense the proximity of other plants and induce shade avoidance responses, but also other phytochromes seem to play a role (Morelli & Ruberti, 2000). Concerning signal transduction pathways, PIF3, a basic helix-loop-helix transcription factor, was identified as one of downstream components which specifically interacts with PHYA and PHYB. The ATHB2 protein is another one. This homeodomain leucine zipper transcription factor acts antagonistically to PIF3. PIF3 mediates the response of phytochrome B to red light and ATHB-2 is strongly induced in FR. ATHB-2 affects auxin response pathways that, on their turn, induce elongation and decreased branching (increased apical dominance) as a response to FR light (shade) (Morelli & Ruberti, 2000).

Constitutive over-expression of phytochrome genes may not be suitable for manipulation of the shade avoidance response (Husaineid, 2007). Phytochrome over-expressing tomato plants only showed a mild suppression of the shade avoidance response. It might be necessary to use tissue specific promoters (Husaineid, 2007).

Phytochrome signal transduction is a highly complex network of events occurring in multiple cellular compartments and little is known about hormonal components involved in regulating the actual growth responses. Not only gibberellins are known to regulate phytochrome-mediated shoot elongation, but also auxins (Pierik *et al*., 2004 and references therein). Phytochrome-mediated shade avoidance responses also involve ethylene action, at least partly by modulating GA action. Plants with inhibited GA production showed hardly any shade avoidance responses (Pierik *et al*., 2004).

In a project of the University of Florida the forage grass Bahia grass was modified over-expressing homeobox-leucine zipper transcription factor AtHB16 from *Arabidopsis*. Enhancing the number of vegetative tillers on expense of reproductive tillers is expected to enhance forage

and turf quality (less lignin)(US notification 07-197-127R). AtHB16 was suggested to play a role as a suppressor of the flowering time sensitivity to photoperiod in wild-type *Arabidopsis* (Wang *et al*., 2003).

3.1.1.4 Genes involved in sugar-signalling

3.1.1.4.1 Trehalose

The trehalose pathway is known to play a central part in the coordination of metabolism with growth and development as part of a signalling network that communicates internal and external cues to coordinate these processes (Paul *et al*., 2008). Trehalose is a disaccharide of two glucose units linked in a α,α-1,1 configuration. In most plants trehalose is hardly detectable, while it is abundantly present in arthropods, fungi, bacteria. Its role in regulating the metabolism seems to be the major function in plants (Paul *et al*., 2008 and references therein).

Trehalose synthesis is a two-step process in which trehalose-6-phosphate synthase (TPS) synthesizes trehalose-6-phosphate (T6P) from glucose 6-phosphate (G6P), followed by a dephosphorylation to trehalose by T6P phosphatase (TPP). Trehalase breaks trehalose down into two glucose units. Several genes code for TPS and TPP. TPS1 is expressed constitutively and synthesizes T6P, other TPSs may have a regulatory function. They are expressed in specific organs like siliques and seeds in Brassicas or are highly regulated by light, sugars, starvation, diurnal rhythms, and cytokinin. The expression of both rice TPPs is under strong regulation by stress such as cold, drought, salt, and ABA —specific for each TPP. *A. thaliana* TPPs are also induced by hypoxia and nitrate (Paul *et al*., 2008 and references therein).

The pathway seems to influence embryo and leaf development, cell division and cell wall synthesis, inflorescence architecture, seedling biomass, adult plant biomass and photosynthesis, sucrose utilization, starch metabolism, and tolerance to abiotic stresses, particularly drought (Paul *et al*., 2008 and references therein). The regulatory function is presumably performed by T6P, regulating the utilization of sucrose while amounts of T6P also respond strongly to sucrose. T6P is formed from sucrose breakdown products via sucrose synthase (SUS) forming UDPG on the one hand and on the other hand glucose-6-phosphate (G6P) that is produced from glucose via invertase and hexokinase (HXK) or via fructose and fructose 6-phosphate (F6P) through SUS, fructokinase, and phosphoglucose isomerase.

High sucrose levels (high assimilation capacity) increase the T6P levels through the constitutive expression of TPS1, and stimulate growth. Starvation and stress (low levels of sucrose) depletes the pool of G6P and UDPG and other hexose phosphates resulting in low T6P levels.

Starch metabolism is one of the targets of T6P. T6P activates ADP-glucose pyrophosphorylase (AGPase), the key enzyme of starch synthesis. Also, starch breakdown is regulated through inhibition of the transcription of starch excess 1 (SEX1) and β-amylase, regulated by the transcription factor ABI4. In leaves, Rubisco activity is increased in TPS overproducing transgenics. The elevated amount of Rubisco is due to increased expression of the transcription factor ABI4 that binds to the promoter of Rubisco and CAB genes. The plants have dark green leaves, whereas TPP over-expressing plants reduce the photosynthetic capacity but try to compensate by expanding their leaves. These plants have large light green leaves and have an increased biomass (Goddijn *et al*., 1997; Goddijn & Van Dun, 1999).
T6P also interferes with the transition to flowering (Van Dijken *et al*., 2004). Ectopic expression of *TPS1* increases inflorescence branching.

The maize RAMOSA3 was identified as a TPP. Patent application by DuPont and Cold Spring Harbor Laboratory WO 2006/074437 describes the use of RAMOSA3 to increase the branching of the tassel and ear of a maize plant. Field trial applications are known with numbers 06-324-108N, 07-065-125N, 08-142-108N and 09-117-107N. In the latter three also *RAMOSA1* is mentioned. RAMOSA1 (RA1) is a small zinc finger TF that acts as a repressor of genes involved in the formation of inflorescence branch meristems. When constitutively over-expressing the *RA1* maize gene in wild type *Arabidopsis*, increased organ size was observed, and in particular in flowers and leaves it was found that this was caused by increased cell expansion (Landoni *et al*.,

2007). The mechanism of RA1 action in maize remains unknown. McSteen (2006) suggests a role in GA signalling.

A rice TPP gene construct was introduced in rice and assayed in a field trial in Delaware, US (06-145-109N; phenotype: altered plant development). VIB and KULeuven Research and Development filed a patent application for using TPS to modulate plant growth and starch synthesis (WO 2007/085483). The activity of a class II TPS is down-regulated by co-suppression, anti-sensing or RNA interference to obtain an increased plant biomass yield, opposite to what would be expected from the above. The proposed TPS has a T6P synthase and T6P phosphatase activity. TPS inactivation turned out to promote CYCD3 and ApL3 (starch synthesis) expression.
In another patent application TPP (WO 2008/071767) a class III TPP is proposed to increase seed yield in rice when driven by a constitutive promoter, such as the rice *GOS2* promoter

Trehalose accumulation obtained in transgenic plants, transformed with trehalose biosynthesis genes leads to an improved abiotic stress tolerance (Ge *et al*., 2008). Trehalose protects proteins and membranes from denaturation by replacing water as it hydrogen bonds to polar residues. During desiccation trehalose forms an amorphous glass structure that limits molecular motion, preventing protein aggregation and free radical diffusion (Paul *et al*., 2008 and references therein).

3.1.1.4.2 Other sugars

Sugars not only are the building blocks in developing plants as they lead to the synthesis of carbohydrates such as starch and celluloses, the synthesis of amino acids and fatty acids and other molecules (metabolic function), they also function as signalling molecules (Smeekens, 2000, Rolland *et al.*, 2006). Photosynthesis and carbon metabolism and allocation are themselves subject to rigorous feedback regulation and a prime target of sugar signalling.

Next to trehalose (see above), a signalling function has been demonstrated for hexoses, mainly fructose, glucose and sucrose. Sucrose specifically controls the translation of basic leucine zipper (bZIP)-type transcription factors and hence the downstream gene targets of these transcription factors. But also regulation on the transcriptional level is seen in *e.g.* sucrose synthase, where the promoter contains sucrose-responsive elements. Other genes are repressed by sucrose.

Hexoses, are sensed by hexose transporters (HXT) and hexokinases (HXK) that phosphorylate hexoses. HXK is involved in repression of photosynthesis genes (Smeekens, 2000; Rolland *et al*., 2006 and references therein). However, also glucose by itself is able to induce *CHS*, *PAL1* and genes encoding AGPase, as well as to repress asparagine synthase (ASN1). The signal transduction cascade involves protein kinases, protein phosphatases, Ca^{2+} and calmodulin. Also, it appears that sugar signalling requires activation of MAP kinases.

Different signalling pathways interact in the regulatory network of plant cells. The cross-talk of the sugar, phytochrome and light systems is one example. Sugars negatively interfere with PHYA signalling. There are extensive interactions between sugar and plant hormone signalling (see Figure 4). In germinating cereal seeds α-amylase genes are induced by GA but sugars override the GA signal and repress gene expression when glucose levels in the embryo exceed the demand. Cytokinins and sugars both regulate the activity of *CYCD3* gene to enable the G1/S transition. Sucrose is also an important element in the triggering of floral development. It induces the meristem identity gene *LEAFY*. In developing plants sugars and ABA activate sink-related genes and they promote *e.g.* tuber development.

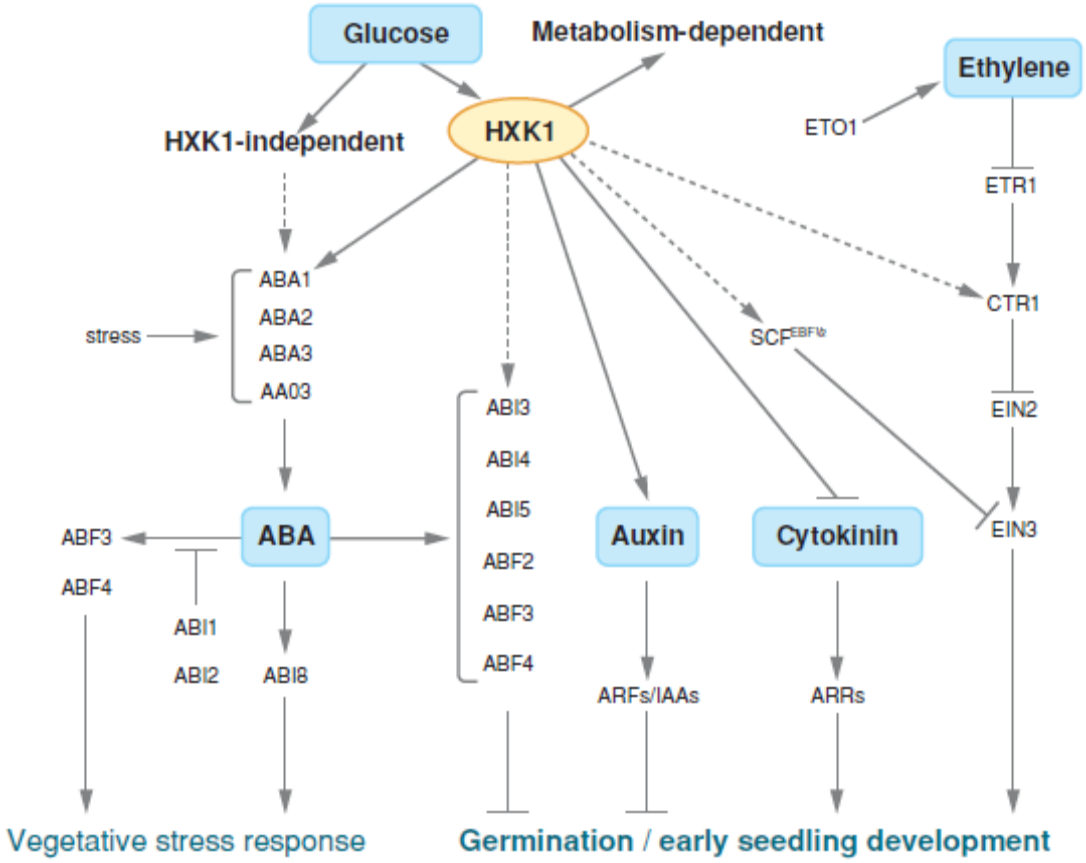

Fig.4 Genetic interactions between sugar and hormone signalling (from Rolland *et al*., 2006; Copyright 2006 Annual Reviews, with permission)

3.1.1.5 Transcription factor genes

RNA polymerases in eukaryotes require the presence of a group of auxiliary proteins to be able to recognize gene promoters. Often these proteins are transcription factors (TFs). TFs are proteins that bind to specific DNA sequences within the promoter or enhancer regions of a gene and in so doing are able to control the transcription of the gene. They may either decrease (repressors) or increase (activators) the level of transcription. They are often involved in the final step in a signal transduction pathway and can be activated or deactivated by other proteins higher in the pathway. TFs are classified according to certain sequence motifs they contain, such as zinc fingers, helix-turn-helix, and basic-helix-loop-helix (for DNA binding) and leucine zippers (for interaction between TFs).

Because TFs naturally act as master regulators of cellular processes, they are expected to be excellent candidates for modifying complex traits in crop plants, and TF-based technologies are likely to be a prominent part of the next generation of biotechnology crops (Century *et al*., 2008).

Mendel Biotechnology Inc, a company in the USA is focussing on TFs. Mendel Biotechnology scientists used essentially all of the TFs genes from *Arabidopsis thaliana* and systematically analyzed the function of each of the encoded proteins by producing experimental plants that had increased or decreased amounts of the target protein. Several patents have been filed claiming large groups of *Arabidopsis* TFs.

The Mendel Biotechnology HERCULES technology which has been demonstrated to increase organ size and biomass in a diverse range of plant species, is based on an AT-hook family TF (Century *et al*., 2008). A number of TFs are involved in leaf senescence, including many from the WRKY, AP2/EREBP, and Myb families.

Mendel Biotechnology discovered an NF-YB transcription factor that confers a stay-green phenotype and an AT-hook transcription factor that promotes biomass production and seed yield (Van Camp, 2005). Patent WO 2004/076638 mentions AT-hook transcription factor genes and OsNF-YB 1 that interacts with a MADS-box protein OsMADS 18. Through a partnership with Monsanto these genes are tested in various crops (Van Camp, 2005). Also CropDesign has several patents filed on TFs and many of these genes are being tested in the field. A large part of these trials are performed in the USA and the nature of the genes is often protected as confidential business information.

In patent WO 2007/064724 several genes - together described as coding for Growth-Related Proteins (GRPs) - are mentioned in strategies to improve yield:
- Seed Yield Regulator (SYR), a protein with some similarity with the *Arabidopsis* ARGOS;
- FG-GAP1; FG-GAP proteins are putative transmembrane proteins;
- CYP90B, involved in BR biosynthesis;
- CDC27, a cyclin-dependent kinase;
- AT-hook transcription factors;
- DOF transcription factors, and
- Cyclin Dependent Kinase Inhibitors (discussed above).

The patent claims that modulating expression of these genes results in improved growth characteristics and increased biomass or seed yield.

SYT1 (synovial sarcoma translocation) is another protein involved in chromatin remodelling. SYT is a transcriptional co-activator which, in plants, forms a functional complex with transcription activators of the GRF (growth-regulating factor) family of proteins. The alteration in local chromatin structure modulates transcriptional activation. SYT1 over-expression in rice leads to increased seed size and seed yield (Van Camp, 2000; WO 2006/079655).

Similarly, over-expression of STZ (salt tolerance zinc finger), a protein involved in stress responses, enhances biomass production and seed yield in rice (Van Camp, 2000; WO 2004/058980). STZ is a zinc finger protein with two zinc finger domains that acts as a transcriptional co-activator.

A Swedish field trial application with hybrid aspen (*Populus tremuloides x Populus tremula*) lists several transcription factors next to other genes to be assessed in field trials (B/SE/09/12395). The genes were selected since they result in increased growth in greenhouse experiments. One *Arabidopsis* orthologue of an aspen gene codes for a GA20 oxidase, the 17 *Populus* genes code for:
- A leucine rich repeat (LRR) protein. Proteins having the LRR motif have diverse unrelated functions;
- A GRAS TF. The GRAS family of TFs have diverse functions in plant growth and development such as gibberellin signal transduction, root radial patterning, axillary meristem formation, phytochrome A signal transduction; and gametogenesis;
- A phosphatidylserine synthase (PSS). Phosphatidylserine synthases are involved in phospholipid biosynthesis;
- A KNOTTED-like homeobox TF 6, 7, 8 and 9;
- Several WRKY TFs;
- A bHLH TF;
- Probably a regulator of the gibberellin response;
- A HD-GLABRA2 TF. GLABRA2 is a homeodomain protein involved in trichome development and also in the regulation of root hair developmen.; and
- A SET-domain protein. The SET-domain protein methyltransferase superfamily includes proteins known to methylate histones on lysine. Histone methylation is important in the regulation of chromatin and gene expression.

3.1.1.6 Other yield enhancing genes

Ste20 is a Ser/Thr kinase belonging to the group of MAP4 kinases (MAP4K). Members of the Ste20 group of kinases are believed to act as regulators of MAP kinase cascades (Dan *et al*.,

2001). In patent application WO 2007/003660 describes claims for an increased seed yield in rice when an *Arabidopsis thaliana* Ste20-like gene is introduced.

In another patent the use of a methionine aminopeptidases (MetAP or MAP), more specifically the *Arabidopsis* MAP2B protein, is mentioned as a way to improve plant harvest (WO 2004/070027). MAPs are responsible for removal of the initiator methionine residue of a peptide during protein synthesis. Methionine aminopeptidases are specific and ubiquitous enzymes that belong to the family of metallo-enzymes. It has been found that eukaryotes have two classes of methionine aminopeptidase (MAP1 and MAP2), while prokaryotes only have one. MAP2 is also known as the eukaryotic initiation factor 2 alpha (eIF2alpha) associated protein p67. It has been demonstrated that rat p67 in addition to its peptidase function, also plays an important role in translational regulation by preventing the phosphorylation of the alpha subunit of initiation factor-2. Accordingly, MAP2 proteins have, in addition to their peptidase activity, a non-proteolytic function to protect eIF2alpha against phosporylation which would make eIF2alpha inactive.

Harvesting oilseed rape requires perfect timing and ideal weather conditions to maximize seed quality and minimize seed loss due to opening siliques. The cell wall between both halves of the siliques is normally degraded as a result of cell wall hydrolase activity in de dehiscence zone. Cell wall hydrolases constitute polygalacturonases and cellulases (β-1,4-glucanases). Anti-sensing an endo-polygalacturonase gene specifically in the dehiscence zone reduces the expression of that enzyme and therefore the siliques do not open at maturity. By prevented seed shattering the crop can stay longer on the field enabling complete maturation of the seeds leading to improved oil quality.

Plant Genetic Systems developed another approach in rapeseed by selectively disabling cells in the dehiscence zone (WO 1997/013865). This was achieved by using genes for products that interfere with cell functions (*e.g.* RNases, ribozymes), with auxin production or ethylene signalling. The decline in auxin during maturation is a major trigger of silique shatter. Maintaining the auxin levels will then prevent dehiscence. Field trials were conducted in four consecutive years (B/BE/99/VW3, B/BE/00/V5, B/BE/01/V5 and B/BE/02/VW4).

Biogemma UK also patented methods to prevent dehiscence of siliques in *Brassicaceae* (WO 1999/015681 and WO 1999/015680). The dehiscence zone 15 (*DZ15*) gene or *OSR7* gene was antisensed to obtain the effect. The function of the gene is not known. In addition another gene *OSR7(9)* is proposed. Its sequence exhibits homology to known sequences encoding xyloglucan endotransferase (XET). XETs are implicated in cell wall loosening and are thus expected to be active in the process of cell wall separation at the dehiscence zone (DZ) which results in dehiscence. Again, reducing the gene's expression will prevent or at least delay seed shatter.

3.1.2 Source-sink: increasing source

Source activities in multi-cellular plants comprise photosynthesis, nutrient mobilization and export. Sink activities are mainly development and storage in storage organs like tubers and seed. There is a tight coordination between the needs of the sink organs and the source activities. The relative strength of different source and sink organs will direct the flow of carbohydrates and other nutrients within the plant and will to a certain extent determine the growth potential of different plant organs. This is accomplished by a complex regulating network including specific sugar-signalling mechanisms (Rolland *et al*., 2006).

3.1.2.1 Photosynthesis / Carbon assimilation

Many attempts have been reported to improve the efficiency of the photosynthesis apparatus either by increasing the capacity per leaf area or by limiting respiratory losses.

One of the methods to change the photosynthetic performance is to modify the plastid number (Dunwell, 2000). Genes controlling this are the plant counterparts of the bacterial cell division gene *ftsZ* (US patent 5,981,836 and US Patent 6,812,382).

ELIP (early light-induced proteins) genes involved in the metabolism of photosynthetic pigments (chlorophylls or carotenoids) were studied in *Arabidopsis* in field trial B/SE/09/2058 (Umeå University).

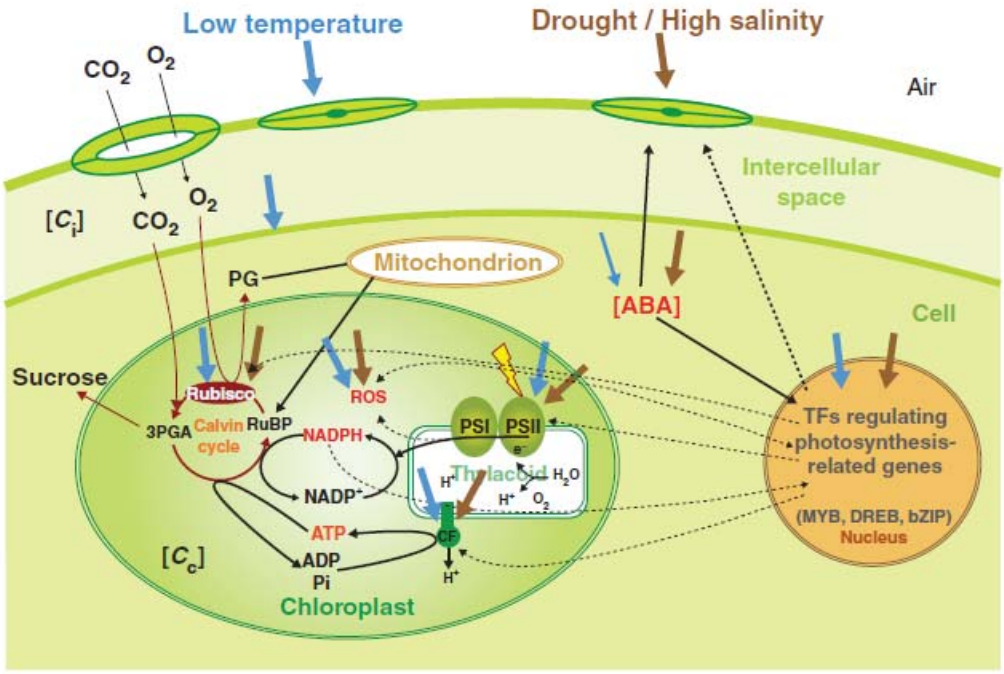

Fig.5 A simplified scheme of the photosynthetic-related mechanisms that can be affected by cold, drought and salinity. Blue and brown arrows correspond to cold and drought/salinity signals, respectively. (from Saibo *et al*., 2009, with permission of Oxford University Press) (PSI, photosystem I; PSII, photosystem II; ROS, reactive oxygen species; 3PGA, 3-phosphoglycerate; RuBP, ribulose-1,5-biphosphate; PG, phosphoglycolate; CF, coupling factor; Ci, internal CO_2; Cc, CO_2 in the chloroplast)

Typically in C_3 plants photorespiration reduces the efficiency. In the C_3-photosynthetic pathway O_2 is inhibiting because of the inherent oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), where O_2 is competing with CO_2 resulting in loss of CO_2. In C_4 plants this is solved by spatial partitioning CO_2 capture and assimilation. Phosphoenolpyruvate carboxylase (PEPC) catches CO_2 in mesophyll cells and passes it to the Rubisco located in the bundle sheet cells. The cell wall limits O_2 passage to the bundle sheet cells and as a result the Rubisco carboxylase activity prevails, reducing losses. In Crassulean acid metabolism (CAM) temporal partitioning is used. PEPC captures CO_2 at night with open stomata, while Rubisco is active during the day with closed stomata. The central idea is then to concentrate CO_2 near Rubisco (Häusler *et al*., 2002; Peterhänsel *et al*., 2008).

PEPC catalyzes the addition of CO_2 to phosphoenolpyruvate (PEP) to form the four-carbon compound oxaloacetate that is further converted to malate and transported to the bundle sheet cells. There CO_2 is released from malate or oxaloacetate by decarboxylation. Rubisco catalyzes the carboxylation of ribulose-1,5-bisphosphate with carbon dioxide to form 2 molecules of glycerate 3-phosphate, which are then used to ultimately synthesize glucose and starch. The Rubisco oxygenation reaction produces single molecules of phosphoglycerate and phosphoglycolate. The latter is recycled into phosphoglycerate whereby CO_2 is released for every two molecules of phosphoglycolate produced (photorespiration).

Attempts to introduce C_4 genes into C_3 plants have thus far been without success (Häusler *et al*., 2002; Peterhänsel *et al*., 2008; Taniguchi *et al*., 2008) as C_4 photosynthesis is closely linked to a

unique leaf anatomy. Although genes similar to the typical C_4 genes are present in C_3 plants, their activity is lower and they probably fulfil other functions in other tissues. Introducing individual genes perturbs metabolism or triggers compensational changes in metabolic fluxes. Over-expressing PEPC in tobacco, rice and potato did induce a higher PEPC-activity, but did not improve CO_2-fixation (see references in Häusler *et al*., 2002). Even transforming rice with 4 C_4 genes did not or not substantially increase CO_2 assimilation (Taniguchi *et al*., 2008).

Using a CO_2-pump to concentrate CO_2 at the site of Rubisco might be an alternative. ICTB, a carbon concentrating protein from *Synechococcus elongatus* mentioned in US soybean trials 09-146-102N and 09-064-105N might be useful. This high affinity bicarbonate transporter gene, *Ict*B, this time from *Cyanobacterium*, was also transferred to rice (Yang *et al*., 2008). The transgenic plants were reported to have 10–30% higher photosynthesis rates and 15–20% higher carboxylation efficiencies. Activities of Rubisco and PEPC were also higher in these transgenic lines. Consistently, the transgenic plants produced 10–120% more tillers or panicles per plant and 10–70% more grains, relative to the wild type (Yang *et al*., 2008).

Kebeish and colleagues (2007) reduced photorespiration or, otherwise said, improved CO_2 recycling, in *A. thaliana* after transfer of the *E. coli* glycolate catabolic pathway to chloroplasts. The net result of this pathway is the formation of glycerate from phosphoglycerate in 4 steps within the chloroplast. This increased biomass production in the transgenic plants concomitant with improved photosynthesis.

In the EU field trial applications B/FR/06/12/06, B/FR/06/01/13, B/FR/05/02/03 and B/FR/03/03/04 by Biogemma a sorghum PEPC is added to the maize genome (both species are C_4 plants) using its own promoter. Biogemma filed a patent on the use of PEPC (WO 2002/081714). Not only yield increase, but also drought tolerance is envisioned. Another way to improve PEPC activity, especially in dry conditions, is the up-regulation of ASR1, a TF that enhances C_4-PEPC and therefore photosynthesis and carbon assimilation (maize, EU application B/FR/05/02/02). *Asr1* is a maize gene and stands for abscisic acid stress ripening.

In the recent patent application WO 2009/016232 PEPC is also mentioned as a target enzyme to modify in order to increase the yield potential of a plant.

Another way to enhance carbon assimilation is increasing the efficiency of Rubisco (Long *et al*., 2006, Peterhänsel *et al*., 2008). One way is to insert a C_4 Rubisco gene into a C_3 plant (US patent 20090172842) or using DNA shuffling to select from Rubisco gene variants the one exhibiting significantly improved carbon fixation activity and CO_2/O_2 specificity (Zhu *et al*., 2004). Another method directs the enzyme Rubisco activase, a key regulator of Rubisco activity. To improve the thermostability of Rubisco activase several thousand variants were screened and the most heat tolerant was picked (Zhu *et al*., 2004).

Enzymes further down, in the Calvin cycle, have also been tackled. Fructose-1,6-bifosfaataldofase (FDA) reversibly catalyzes the reaction converting triosephosphate into fructose-1,6-bisphosphate. Transgenic plants were generated that express the *E. coli fda* gene in the chloroplast to improve plant yield by increasing leaf starch biosynthetic ability in particular and sucrose production in general (US Patent 6663906). Transgenic plants also had a significantly higher root mass. Furthermore, transgenic potatoes expressing FDA exhibited improved uniformity of solids.

Soybean was transformed with the fructose-1,6-/sedoheptulose-1,7-bisphosphatase gene from the cyanobacterium *Synechococcus* and tried in the field (US notifications 09-146-102N and 09-064-105N). The same type of experiments in tobacco revealed that over-expression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase raised final dry matter and photosynthetic CO_2 1.5-fold and 1.24-fold, respectively (Miyagawa *et al*., 2001). Transgenic tobacco also showed a 1.2-fold increase in initial activity of Rubisco compared with wild-type plants. Over-expressing an *Arabidopsis* sedoheptulose-1,7-bisphosphatase gene in tobacco was shown to increase the activity of the whole Calvin cycle (Lefebvre *et al*., 2005). Photosynthetic rates were increased, higher levels of sucrose and starch accumulated during the photoperiod,

and an increase in leaf area and biomass of up to 30% was also seen. The gene was also mentioned in a tobacco trial application in the US (09-089-112N).

In aging leaves several changes occur involving the degradation of macromolecules and re-cycling of regained nutrients from basal leaves to the actively growing parts of the plant (leaf, stems and seeds). The most visible event is the yellowing of the leaf due to the breakdown of chloroplasts. Senescence occurs both as an intrinsic process controlled by developmental signals such as aging and hormones or in response to environmental stresses, such as nutrient deficiency, low light intensity, drought or pathogen attack. Preventing senescence prolongs the time span over which a leaf contributes to the photo-assimilation of a plant.

Since Gan and Amasino (1995) explained their strategy to delay leaf senescence using a senescence regulated promoter coupled to the isopentenyltransferase (IPT) gene, many crops have been modified expecting that cytokinins will inhibit senescence (Calderini *et al.*, 2006; Li *et al*., 2004). Expression of the *IPT* gene, involved in cytokinin synthesis, specifically in aging leaves, keeps the chloroplasts active. Several promoters have been used not only to delay senescence in leaves, but also to improve shelf life of fruits and flowers in horticultural produce (US Patent 7,227,055; Li *et al*., 2004).

As leaf senescence is also a response to stress, delaying this phenomenon will have an effect on drought and heat tolerance. Field trial applications in the US therefore also indicate these tolerances as the expected phenotype. In notification 09-103-109N an *Agrobacterium ipt* gene was inserted in peanut in connection with genes typical for obtaining drought tolerance and for root formation to increase yield. Also tobacco has been modified with a bacterial *ipt* for drought tolerance (US application 08-088-104N) and creeping bent grass for heat tolerance (US application 06-305-01R).

Another method to inhibit senescence in photosynthetic organisms is the inhibition of farnesyl transferase activity. The resulting plants stay green and tissue viability is maintained for a longer period of time (patent WO 99/006580). This enzyme is involved in ABA signal transduction. The Max Planck Institute for Chemical Ecology uses *N. attenuatta* to study a large amount of genes, among which also the genes for farnesyl pyrophosphate synthase, Rubisco, Rubisco activase, phytochromes, sedoheptulose-1,7-bisphosphatase, etc.(US application 06-242-03R).

Also, senescence and shade avoidance responses are related. By repressing the shade avoidance reaction in field crops, the lower leaves still receive enough light to suppress the degradation of chloroplasts.

Despite the sometimes disappointing results in relation to photosynthesis, research goes on as seen with the Umeå University studying the photosynthetic system in *Arabidopsis* (B/SE/04/1310, B/SE/08/2142 and B/SE/09/2058). Recently BASF entered a research cooperation with the University of Cologne to increase yield and to improve the crops' tolerance to adverse environmental conditions, more specifically to optimize CO_2 use[9]. Also BayerCropSciences collaborates with the University of Technology[10], Aachen, to reduce CO_2 loss due to photorespiration.

3.1.2.2 Nitrogen uptake and assimilation

In addition to modifying photosynthesis to increase the capturing of light energy, the use of genes involved in nitrogen metabolism has also been a successful approach to increasing biomass (Ragauskas *et al*., 2006). If photosynthesis is increased, the next limiting factor is nitrogen assimilation. Carbon and nitrogen metabolism are tightly linked and it seems obvious that nitrogen signalling pathways interact with sugar signalling pathways (Smeekens, 2000; Rolland *et al*., 2006).

[9] http://www.basf.com/group/corporate/en/function/conversions:/publish/content/products-and-industries/biotechnology/images/PI_BASF_Provendis_e.pdf
[10] http://www.research.bayer.com/edition-19/19_Photosynthesis.pdfx

'Nitrogen-use efficiency' (NUE) is the efficiency with which the plant uses nitrogen from the soil and is composed of two components:
1) uptake efficiency; and
2) assimilation efficiency.
NUE can be expressed as yield of grain (or harvestable product) per unit of available nitrogen in the soil, or as the relative balance between the amount of fertilizer nitrogen taken up and the amount used by the crop versus the amount of fertilizer nitrogen "lost". Another way of expressing NUE is in terms of the number of kg of grain harvested versus kg of applied nitrogen.

Roots take up nitrogen as nitrates and ammonium. The high affinity nitrate transporters NRT2 and the low affinity nitrate transporters NRT1 are induced by nitrate (Lea & Azevedo 2006; Stitt, 1999 and references therein). Ammonium is taken up by the family of ammonium transporters (AMT). In the plant cell nitrate is reduced to ammonium by nitrate reductase (NR) and nitrite reductase. Ammonium is further assimilated via the glutamine-oxoglutarate aminotransferase (GOGAT) pathway by the enzymes glutamine synthetase (GS2, GS1) and glutamate synthase. The recycling of nitrogen released in metabolism, *e.g.* ammonium released in photorespiration and in protein breakdown, is also important in the nitrogen economy of a plant. Glutamine synthetase is thought to play a key role herein.

Nitrogen assimilation requires not only inorganic nitrogen but also the carbon skeleton 2-oxoglutarate (2-OG) that is produced through sequential reactions from photoassimilated carbohydrates (Figure 6). The levels of carbon and nitrogen metabolites mutually influence each other, implying the intimate link between carbon and nitrogen metabolisms (Yanagisawa *et al*., 2004 and references therein). Nitrate, ammonium, or amino acids may also serve as signalling molecules in plants (Coruzzi and Bush, 2001).

NR is thought to be the most limiting step in nitrogen assimilation and is regulated transcriptionally as well as translationally, the reason why modifying this gene not always results in the intended effect (Lea & Azevedo, 2007; Shrawat & Good, 2008).

The high affinity nitrate transporters NRT2 presumably have also a role in the regulation of root branching.

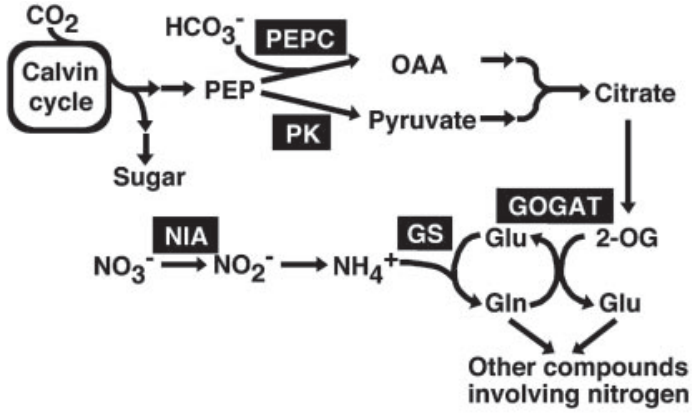

Fig.6 The metabolic pathway for nitrogen assimilation in plants (from Yanagisawa *et al*., 2004 Copyright 2004 National Academy of Sciences, U.S.A., with permission). Abbreviations: GS, glutamine synthetase GOGAT, glutamate synthase; NIA, nitrate reductase; OAA, oxaloacetate; PEP, phosphoenolpyruvate

Enhancing NUE may be achieved by introducing genes encoding nitrogen transporters, nitrate and nitrite reductases, glutamine synthetase and synthases, aminotransferases and dehydrogenases, and transcription factors. Some examples:
- Patent application WO 2009/095455 describes the introduction of a diatom high affinity nitrate transporter 2 (NRT2) polypeptide;

- WO 2007/051866 includes NTR genes;
- WO 2009/065912 takes an AMT to enhance ammonium uptake in rice plants using the rice GOS2 promoter;
- Ceres Inc. filed a patent (WO 2009/105492) listing sequences of nucleic acids for improved NUE. Two constructs 35S::NRT1.3 and 35S::NRT1.4 introduced in *Arabidopsis* were given as an example;
- WO 2009/016212 mentions nitrite reductase or an asparagine synthase as the target enzymes to modify their expression;
- Patent application WO 2009/080743 proposes to repress several genes to enhance NUE and improve yield (*Arabidopsis* genes with unknown or partially known functions); and
- Patent application WO 2009/037279 claims to have a method to enhanced NUE and/or increased biomass production using one or more genes from a huge list.

Glutamine synthetase (GS) occurs in two forms: cytosolic GS (GS1) encoded by a complex multigene family, and plastidic (GS2) encoded by only one gene. In a 3-year field trial of transgenic poplar (*P. tremula* x *P. alba*) over-expressing a *GS1* gene, tree height increased to 141% of control plants by the third year of the study (EU application B/ES/98/27; Jing *et al*., 2004). The poplar plants were transformed with a chimaeric gene construct containing the CaMV 35S promoter and a pine *gs1* (Gallardo *et al*., 1999; Jing *et al*., 2004). GS1 is thought to be involved in primary assimilation in roots and in the reassimilation of ammonium released in other metabolic processes. In the photosynthetic organs of angiosperms, GS1 expression is restricted to vascular bundles, indicating a role in the transport of glutamine from the leaf to other organs. GS2 acts in coordination with ferredoxin-dependent glutamate synthase (Fd-GOGAT) to assimilate ammonium derived from nitrate reduction and photorespiration. In conifers GS1 is expressed in photosynthetic cells, where GS2 is absent. In the GM poplar plants the pine GS1 is now expressed also in mesophyll cells adding to the endogenous GS2. The field trial analyses support a higher capacity of transgenic trees, not only in primary nitrogen assimilation but also in the reassimilation of ammonium released in different metabolic processes (Jing *et al*., 2004).

The potential of GS1 for engineering biomass increase is further emphasized by results showing that quantitative trait loci for yield in maize and maritime pine map to the location of GS1 (Rasgauskas *et al*., 2006). Similar possibilities are evident in the over-expression of a bacterial glutamate dehydrogenase, which increased the biomass of tobacco plants under both laboratory and field conditions (Good *et al*., 2005; Ragauskas *et al*., 2006).

Also in wheat (EU application B/ES/02/16) and maize (EU applications B/FR/03/02/04 and B/FR/05/01/01) a glutamine synthetase has been used. In the maize study a cytosolic maize glutamine synthetase (GS1), product of the *Gln1-3* gene, was investigated (Martin *et al.*, 2006). Constitutive over-expression increased the kernel number with 30% in a greenhouse under suboptimal nitrogen conditions.

Especially under nitrogen starvation the beneficial effects of the transgenics are visible. Tobacco transformants over-expressing alfalfa GS1 driven by the CaMV 35S promoter had ±70% higher shoot and ±100% more root mass (dry weight) (Fuentes *et al*., 2001). The level of photosynthesis under nitrogen deficiency was for the transformants the same as in control plants grown under high nitrogen conditions. The authors suggest a role in reassimilation of photorespiratory ammonia now that GS1 is also expressed in mesophyll cells. Or, it might be that over-expression of GS1 during leaf senescence increases the sink strength of young developing plant parts remobilizing and reassimilating ammonia from protein breakdown in older leaves.

The effect of over-expressing GS1 differs depending on the species and tissue in which it is expressed (Fei *et al*., 2006; Good *et al*., 2004). Transgenic pea plants over-expressing a soybean cytosolic *GS15* gene under the control of a root specific promoter also demonstrated an increased biomass and nitrogen content (Fei *et al*., 2006).

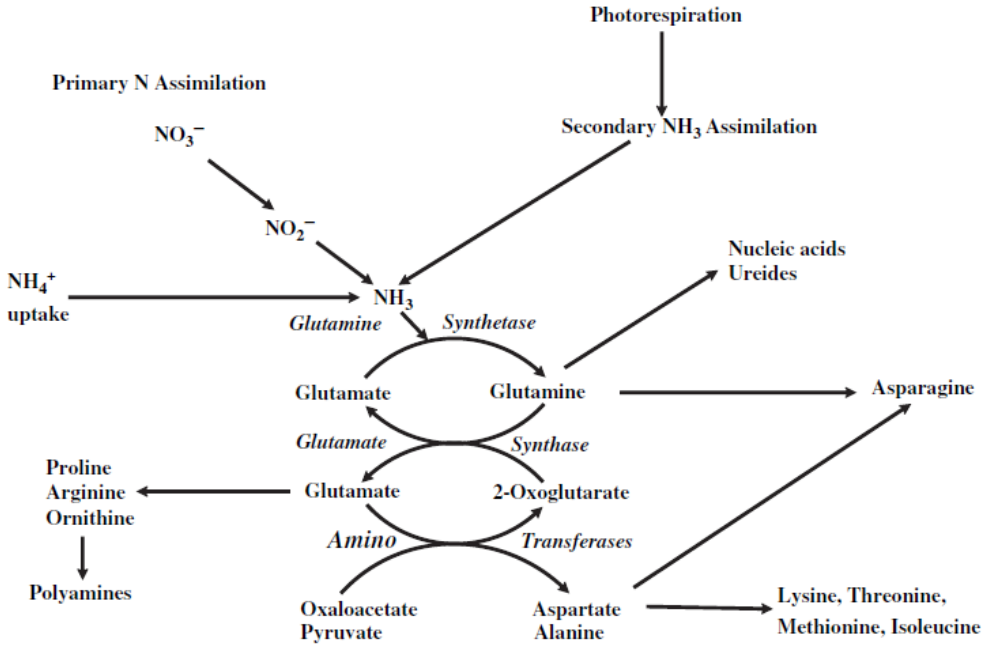

Fig.7 The pathways of the assimilation of nitrate and ammonium into amino acids (from Lea & Azevedo, 2007; Copyright 2007 John Wiley & Sons, with permision).

Glutamate synthase (GOGAT) appears in two isoforms: ferridoxin and NADH-dependent, both of which are located in the plastid. Yamaya and colleagues (2002) over-expressed OsNADH-GOGAT1 in rice under the control of its own promoter and found that transgenic rice plants show an increase in spikelet weight (up to 80%).

The nitrogen element in glutamate and glutamine can be transferred to a wide variety of amino acids, nucleic acids, ureides and polyamines (Lea and Azevedo, 2007 and references therein).

Alanine is such an amino acid (see Figure 7). Alanine aminotransferase (AlaAT) catalyses the reversible transfer of an amino group from glutamate to pyruvate to form 2-oxoglutarate and alanine. The regulation of *AlaAT* in several plant species has been studied in response to low-oxygen stress, light and nitrogen application. The different forms of the enzyme can be located in the cytosol, mitochondria and peroxisomes.

Good and colleagues (2007) constructed oilseed rape plants expressing an *AlaAT* gene isolated from barley encoding a cytosolic form of alanine aminotransferase under the control of a *btg26* promoter. The *btg* gene encodes a protein antiquin, which is induced by drought and temperature stress, high salt and abscisic acid. Grown in a low nitrogen hydroponic system, increases of 55–64% in biomass were observed when compared with wild-type plants or transgenic plants in which the *AlaAT* gene was under the constitutive control of the CaMV 35S promoter. When the *btg-AlaAT* plants were grown in the field at suboptimal rates of nitrogen application, there was a 42% increase in seed yield.

The University of Alberta, Canada patented the invention (U.S. Patent No. 6,084,153). In the US 7 field trial applications were counted in the past 5 years (05-262-02N, 06-087-11N, 06-254-102N, 07-116-101N, 07-253-101N, 09-047-112N and 09-267-103N). Arcadia has fine-tuned the technology developed by the University of Alberta, to develop and test NUE in cereal crops (WO 2007/076115). In rice, as a model monocot crop, this strategy is being studied (US applications 06-090-20N, 07-071-103N, 07-110-101N, 08-029-107N, 08-086-104N and 09-064-104N). Early research in rice showed that, with NUE technology, a half-rate of nitrogen still results in higher tiller count and increased panicle number. Arcadia has licensed NUE technology to a number of companies in several crops, including Monsanto for canola and Pioneer for corn.

It also collaborates with Vilmorin, France to develop NUE wheat and with Advanta, India for NUE sorghum.

Glutamate:glyoxylate aminotransferase (GGAT) catalyses the reaction: glyoxylate + L-glutamate <=> glycine + 2-oxoglutarate. This enzyme participates in glycine, serine and threonine metabolism. Patent application WO 2009/013225 uses a root-specific promoter to over-express this enzyme specifically in root tissue. The resulting transgenic plants grow better under low nitrogen conditions.

WO 2009/013263 mentions an alanine aminotransferase gene or an alanine aminotransferase-like gene or genes for an AT-hook motif nuclear localized 19/20 polypeptide and a metallothionein 2a polypeptide for better NUE.

Instead of over-expressing the enzyme of choice itself, it may also be modified using transcription factors influencing the gene's expression. *ZmDOF1* from maize is encoding a DNA binding factor with one zinc finger and seems to regulate the coordinated expression of a set of key genes in the carbon and nitrogen assimilation pathways (Yanagisawa *et al*., 2004). *Arabidopsis* plants expressing DOF1 showed an increase in amino acid content, along with enhanced nitrogen assimilation under normal conditions and better growth under low-nitrogen conditions. Expression of PEPC, pyruvate kinase, citrate synthase and isocitrate dehydrogenase genes was seen to be up-regulated (Yanagisawa *et al*., 2004). The invention was patented by Ajinomoto Co., Inc., Japan (US patent 7,176,351) and examples were given for potato using a CaMV 35S::maize *DOF1* gene construct. The potatoes yielded more when grown under normal conditions.

The same gene was transferred to sugarcane using the maize ubiquitin promoter and tested in the field (Australian applications DIR070/2006 and DIR095/2009). In the first trial plant height was increased with approximately 20%, with a small decrease in stalk number and stalk diameter. Final cane yield increased with approximately 20%.

Patent application WO 2009/056566 also illustrates the use of a *DOF* gene, *DOF-C2*, or a MYB7 transcription factor to increase yield related traits. Constitutive or seed-specific promoters are used. No mentioning of stress conditions is made.

In another Australian field trial wheat and barley received a gene indicated as *Me1*, a metabolic enzyme gene from barley expected to enhance NUE (Australian application DIR 094/2009 and DIR 099/2010). However, the exact function is not disclosed. This time a tissue specific promoter is used that is not expressed in the grain. The same gene has been expressed in oilseed rape and rice and the resulting plants showed greater biomass and higher yield than control plants (RA DIR 094/2009).

In patent application WO 2008/132231 a rice class I homeodomain leucine zipper hox5 transcription factor is described as having positive effects on rice growth under reduced nutrient conditions (constitutive over-expression). No mechanism was proposed.

Ceres Inc.'s patent WO 2009/105612 has a long list of gene sequences for proteins containing a P450 domain, or for aminotransferases, glutamate decarboxylase, amino acid transporters and amino acid permeases, or proteins belonging to the linker histone HI and H5 family, RING finger protein family, AN1-like zinc finger domain protein family, A20-like zinc finger domain protein family and many others. Their function is often not known. Examples are given for *Arabidopsis*.

3.1.2.3 Phosphate uptake

The next essential element that plants retrieve from the soil is phosphate. It is important for building nucleic acids and cell membranes, energy metabolism (ATP), enzyme regulation and signal transduction.

Florigene, Australia transformed the ornamental torenia (*Torenia x hybrid*) with *PHR1*, an *Arabidopsis* phosphate starvation response regulator 1 gene, to enhance phosphate uptake. The

PHR1 gene encodes a transcription factor thought to play a role in plant responses to phosphate deficiency. This response involves a modification of the root system (root architecture), increased mobilization of phosphate from organic sources (secretion of hydrolases and phosphatases) and modification of phosphate transport (expression of high affinity transporters; redistribution from senescing tissue) (Nilsson *et al*., 2007; Australian application DIR 084/2008 and references therein). Expression of PHR1 is independent from the phosphate status but is increased during phosphate starvation, probably by post-translational regulation. In greenhouses it turned out that torenia plants constitutively expressing *PHR1*, depleted phosphate from hydroponic media more rapidly. This was also tested in *Arabidopsis* (Nilsson *et al*., 2007). Over-expression resulted in enhanced accumulation of phosphate in the shoots.

Patent application WO 2009/020528 claims that over-expression of a plant vacuolar pyrophosphatase also increases phosphate uptake under phosphate-sufficient growth conditions next to salt tolerance resulting in an increased yield and larger plant size.

Phosphate assimilation is clearly linked with carbohydrate metabolism.

3.1.3 Source-sink: increasing sink

Carbohydrate metabolism has been modified both in source tissues, with the aim of increasing the supply of metabolites to heterotrophic sink organs, and in sink tissues to enlarge the sink capacity (Van Camp, 2005). In the vegetative phase of a plant's life cycle young shoot and root tissues serve as a sink. In the generative phase the developing flowers and seeds or other storage organs like tubers or roots are the sink organs. Assimilates are either immediately directed to the sink organs or they are recycled due to remobilization of starch and proteins from leaves and roots.

In sink tissues, sucrose can be imported into cells through plasmodesmata (symplastic transport) or the cell wall (apoplastic transport). Intracellular sucrose is cleaved by cytoplasmic invertase (C-INV), generating glucose and fructose, or by sucrose synthase (SUS) producing fructose and UDPglucose. In the apoplast, extracellular sucrose is hydrolysed by cell wall invertase (CW-INV), a major driving force in sugar unloading and gradient maintenance and therefore sink strength. These enzymes generate high levels of extracellular glucose and fructose that are taken up by HXTs, which are co-expressed and co-ordinately regulated with CW-INV (Rolland *et al.*, 2006 and references therein).

3.1.3.1 Starch biosynthesis

ADP-glucose pyrophosphorylase (AGPase) produces the substrate for starch synthesis and is thought to catalyse a rate-limiting step in the starch biosynthetic pathway. AGP catalyzes an important regulatory step that produces ADP-Glc and pyrophosphate (PPi) from glucose-1-phosphate (G1P) and adenosine 5' triphosphate (ATP). The resulting ADP-Glc molecule serves as an activated glucosyl donor during α-1,4-glucan synthesis. AGPase, a heterotetramer, is highly regulated by sugars. Leaf AGPase is activated in the light and inactivated at night. AGPase activity is normally located in the plastids except for cereal endosperm where it is cytoplasmic and plastidic, the cytoplasmic being the most important fraction. Maize shrunken2 (*sh2*) and brittle2 (*bt2*) mutants are the result of mutated AGPase subunits. Trehalose stimulates the expression of AGPase and starch synthesis in *Arabidopsis* (Wu, 2008 and references therein).

Wheat and rice have been modified with adding extra AGPase. In wheat a modified maize *Sh2* gene with its own endosperm-specific promoter encoding an AGPase large subunit exhibits increased AGPase activity resulting in 38% more seed weight per plant and 31% more biomass (Smidansky *et al*., 2002). The same gene construct in rice increased seed weight and plant biomass by more than 20% (Smidansky *et al*., 2003). These research groups applied for wheat field trials under US notifications 05-032-12N, 05-049-04N, 05-049-05N and 06-073-07N and for maize under 05-195-06N (only the most recent notifications are mentioned). The inserted gene was from maize. Many other field trials with this gene have been conducted since 1999 for

maize, rice and wheat. Michigan State University inserted an *E. coli* AGPase gene construct in potato and trialled under US notification 06-150-101N.

The University of Cologne introduced in potato the *gpt* (glucose-6-phosphate/phosphate translocator) gene of pea and the *ntt1* (adenylate translocator 1) gene of *Arabidopsis* (EU application B/DE/05/175). The genes were controlled by the B33 patatin promoter which drives expression of genes mainly in potato tubers. The GPT protein transports G6P in counter exchange with phosphate or triose phosphate across the inner envelope membrane of plastids. NTT is an adenylate translocator that transports ATP in counter exchange with ADP across the inner plastid envelope membrane. With the patatin promoter more G6P and ATP is imported in the amyloplast where starch synthesis takes place and optimal AGPase activity is ensured. In greenhouse experiments the double transformants showed a higher tuber yield (19% more) and higher tuber starch content (up to 28% more) (Zhang *et al*., 2008). Single over-expression of either gene did not have that effect. The final report of the 2-year field trial, however, mentions the same or lower starch level and a slightly lower tuber yield as compared to control plants. The tubers could grow at the expense of berries indicating a higher sink strength.

Apyrases (nucleoside triphosphate-diphosphohydrolase) are enzymes that can hydrolyze nucleoside triphosphates (NTPs) and/or diphosphates (NDPs). They are found in all eukaryotes and are far more efficient in removing phosphates from NTP than other phosphatases. The majority of characterized apyrases are ectoapyrases, *i.e.* enzymes that are anchored in the plasma membrane with their active site pointing out into the extracellular matrix.

Potato apyrase has additionally been speculated to be involved in starch synthesis. A special feature of potato-specific apyrase is that it is soluble and does not appear to be strongly bound to membranes, but is confined to the apoplast (Riewe *et al*., 2008). Potato apyrase1 activity has been repressed using an RNAi construct driven by the B33 patatin promoter for field trials in Germany (EU application B/DE/04/157). Under greenhouse conditions the modification resulted in a higher tuber yield. Tubers grew more longitudinally. A decrease in apyrase activity did not directly perturb major metabolic processes, but led to specific changes in the expression of nuclear genes encoding extensions. This may explain developmental alterations, since they are suggested to play a role in polar growth (Riewe *et al*., 2008). Probably, the apoplastic ATP levels and changes therein affect a signalling pathway. Although the field trial application intended trials for 5 consecutive years, potatoes were only planted in 2005 and 2006.

In field trial applications in the US sucrose phosphate synthase (SPS) and sucrose synthase (SUS) are indicated to enhance yield in rapeseed and maize respectively. SPS catalyses the reaction from fructose-6-phosphate (F6P) and uridine 5'-diphosphate glucose (UDP-glucose) to sucrose-6-phosphate (S6P) in the cytosol.

Increased starch content may also be obtained by over-expressing a gene of the pyrimidine metabolism (patent application WO 2001/014569). Dihydroorotase is an enzyme that converts carbamoyl aspartic acid into 4,5-dihydroorotic acid, one of the steps in the synthesis of UDP. UDP-glucose is needed to make sucrose that is then transported to the sink organs where it is cleaved into fructose and glucose the building block of starch. Combined with a constitutive or leaf-specific promoter and a plastid targeting sequence, this gene is claimed to increase potato starch content.

The US Patent 7,176,351 mentioned earlier describing potatoes having an increased yield of starch per plant also fits in this chapter.

Bayer CropScience recently applied for field trials in Brazil with sugarcane of which the carbohydrate metabolism has been altered (Brazil application: 01200.002916/2009-57, 01200.002944/2009-74). No gene names were disclosed.

Again, the IPT protein, that catalyses the first reaction in the biosynthesis of isoprene cytokinins, may be used to increase sink strength. Cold Spring Harbor Laboratory expressed the maize *IPT2* gene ectopically in maize and organized field trials (US notifications 07-065-125N, 08-142-108N,

and 09-117-107N). Although many other genes are mentioned in the applications, *ZmIPT2* is described as a preferred gene to increase seed yield using genetic modification techniques (Brugière *et al.*, 2008). Cytokinin levels are increased during normal kernel development and they might influence the unloading of photoassimilates up-regulating extracellular invertase (Brugière *et al.*, 2008 and references therein).

3.1.3.2 Oil biosynthesis

Storage lipids in seeds are synthesized from carbohydrate-derived precursors. The molecules G6P and pyruvate are transported into the plastids and converted into acetyl-CoA that serves as the primary precursor for the synthesis of fatty acids.

- Using a seed-specific promoter the gene for glycerol-3-phosphate dehydrogenases (G3PDH) from *Saccharomyces cerevisiae* induces a total oil level increase in transgenic *Arabidopsis* (patent application WO 2003/095655);
- A gene for a yeast triacylglycerols synthesis enhancing protein was used to increase total oil content in *Arabidopsis* (patent application WO 2004/007727);
- In patent application WO 2009/027335 methods are described to increase the oil content in oil crops by up or down-regulating proteins that are involved in lipid biosynthesis or breakdown. Examples are lipases, chlorophyllide A oxidases, 14-3-3 proteins, ABC transporter proteins etc. The gene constructs contain seed-specific promoters and plastid signal sequences; and
- Another way to increase oil levels is diverting assimilates and energy from biosynthesis of storage proteins to lipid biosynthesis (WO 2003/077643). This is done by down-regulating the gene for one or more storage proteins.

3.2 Stress tolerance

Munns & Tester (2008) defined stress as an adverse circumstance that disturbs, or is likely to disturb, the normal physiological functioning of an individual. It is an influence that is outside the normal range of homeostatic control in a given genotype. Where a stress tolerance is exceeded, response mechanisms are activated (Lerner, 1999) requiring energy. Where the stress is controlled a new physiological state is established, homeostasis is re-established. When the stress is removed the plant may return to the original state or a new physiological state may be established (Amzallag, 1999).

Two types of stress factors are commonly distinguished:

- abiotic stress factors: salt, drought, flooding, heat, cold, toxic substances (heavy metals), and
- biotic stress factors: pest and diseases, weeds.

This survey is limited to abiotic stress tolerance.

The genes in this chapter are again organized according to their type of action, rather than the aimed trait. The complex plant response to abiotic stress can be subdivided into processes where genes are involved

(i) in signalling cascades and in transcriptional control,
(ii) in the protection of membranes and proteins, and
(iii) in water and ion uptake and transport (Wang *et al*., 2003).

This cascade of events is illustrated in Figure 8.

Primary stresses, such as drought, salinity, cold, heat and chemical pollution are often interconnected, and cause cellular damage and secondary stresses, such as osmotic and oxidative stress. The initial stress signals (*e.g.* osmotic and ionic effects, or temperature, membrane fluidity changes) trigger the downstream signalling process and transcription controls which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes (Wang *et al*., 2003). Attempts to obtain plants with an increased abiotic stress tolerance involved modification of genes in either of these processes.

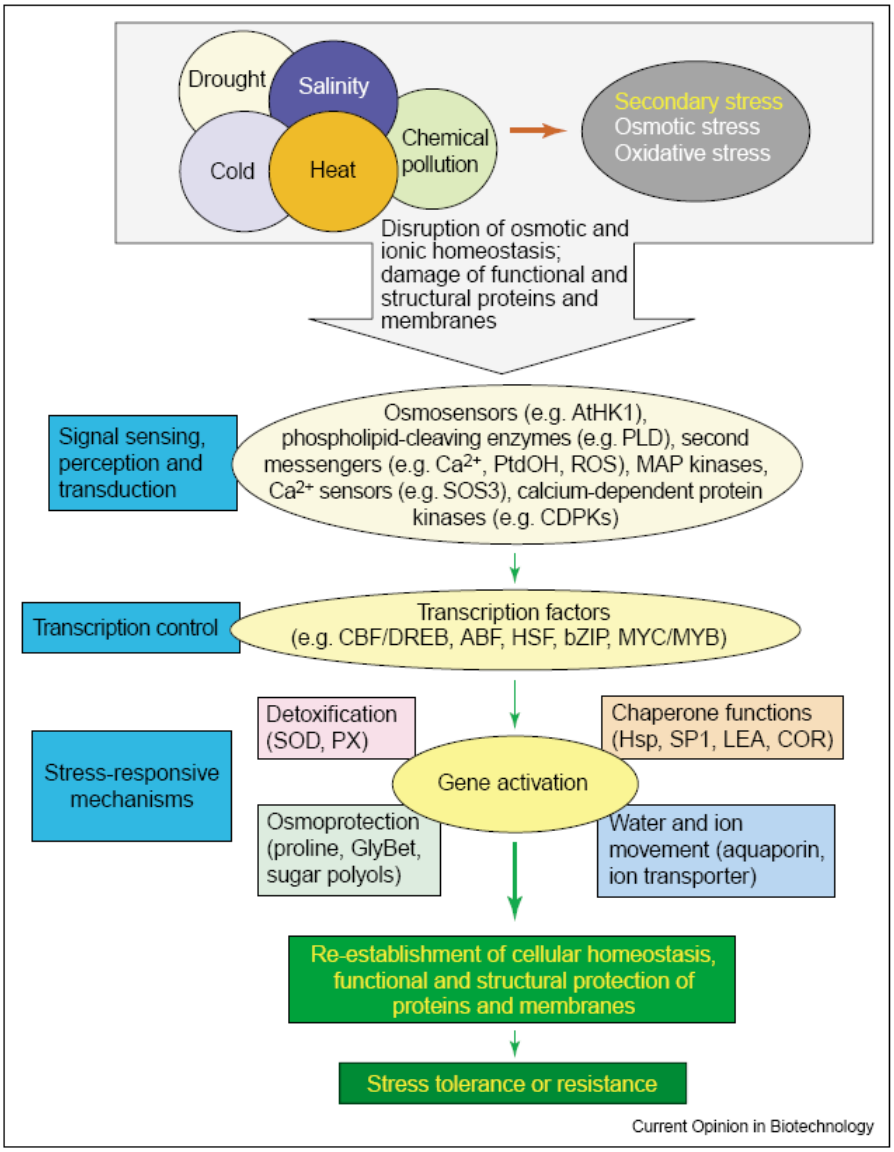

Fig.8 The complexity of the plant response to abiotic stress (from Wang *et al*., 2003; Copyright 2003 Springer, with permission).
Abbreviations: ABF, ABRE binding factor; AtHK1, *Arabidopsis thaliana* histidine kinase-1; bZIP, basic leucine zipper transcription factor; CBF/DREB, C-repeat-binding factor/dehydration-responsive binding protein; CDPK, calcium-dependent protein kinase; COR, cold-responsive protein; Hsp, heat shock protein; LEA, late embryogenesis abundant; MAP, mitogen-activated protein; PLD, phospholipase D; PtdOH, phosphatidic acid; PX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; SP1, stable protein 1.

Sometimes WUE is used as a synonym of resistance to drought. However, while WUE may be expressed as unit of biomass produced per unit of water used by the plant, it is a breeding target also applicable to optimal growing conditions. Physiologically it is a ratio between photosynthesis and transpiration. WUE depends on the stomatal conductance to CO_2 versus to H_2O and the concentration gradient of either of them between the air inside and outside the leaf. Nevertheless, plant varieties with a higher WUE may also better withstand dry environments.

The expression patterns of genes induced by drought, high-salinity, and cold stresses in *Arabidopsis* reveal that of the 300 genes that have been identified more than half of the drought-inducible genes are also induced by high salinity, indicating the existence of significant cross-talk between the drought and high-salinity responses (Yamaguchi-Shinozaki and Shinozaki, 2006

and references therein). By contrast, only 10% of the drought-inducible genes were also induced by cold stress.

Abiotic stress causes an over-excitation of the light reactions of the photosynthetic system. When electron transport exceeds the requirements of normal metabolism, electrons accumulate in the electron transport chain and, if not removed by other processes (xanthophyll cycle, photorespiration), they are transferred to oxygen. Such processes generate reactive oxygen species (ROS), *e.g.* superoxide (Chaves & Oliveira, 2004; Saibo *et al*., 2009). ROS cause peroxidation and de-esterification of membrane lipids, and also lead to protein denaturation.

Drought, salinity and cold induce stomatal closure, slowing down the CO_2 diffusion and as a result the photosynthetic rate reduces. Opening and closing of the stomata is mediated by ABA-dependent and independent signalling. When photosynthesis goes down, likewise the Calvin cycle's activity is reduced and therefore the concentration of the electron acceptor of the photosystem $NADP^+$ lowers, leading to an excess of excitation of the photosystem and ROS. High and low temperatures also slow down enzyme activity, like Rubisco and Rubisco activase and other Calvin cycle enzymes (Chaves & Oliveira, 2004; Saibo *et al*., 2009 and references therein).

Abscisic acid (ABA) is produced under water-deficit conditions and plays an important role in the tolerance response of plants to drought and high salinity among which stomatal closure. The role of ABA in cold stress-responsive gene expression is not clear (Yamaguchi-Shinozaki and Shinozaki, 2006).

Abiotic stress induces premature senescence which results in leaf chlorosis, necrosis, defoliation, cessation of growth and reduced yield. Cytokinins counteract many of processes mediated by ABA such as the closure of stomata and the acceleration of senescence. By increasing the production of cytokinins plants might recover better from stress. Harding and Smigocki (1994) found that transgenic *N. plumbaginifolia* plants with an *IPT* gene behind a heat shock promoter show upon induction a rapid increase of mRNA of several genes among which stress-related genes. In the same way as the addition of the *Agrobacterium IPT* gene driven by a senescence specific promoter to the genome may postpone the natural senescence of a plant, it prolongs the stay green period in stress conditions. This was confirmed in *Arabidopsis* exposed to flooding (Zang *et al*., 2000). Transgenic tall fescue plants containing an *Agrobacterium IPT* gene show enhanced tolerance to low temperatures (Hu *et al*., 2005). The plants have higher chlorophyll levels and a longer stay-green period under low temperature conditions. Creeping bent grass has been modified with the same gene, but this time heat tolerance was envisioned (US application 06-305-01R). Also, cotton (US application 09-093-113N) and tobacco (US application 08-088-104N) were transformed with the bacterial gene, but here the aim was to induce drought tolerance.

Several companies have filed patents on the subject of abiotic stress. Some examples include:

- Monsanto's patent US 2006/236419 A1 claims thousands of sequences to improve plant cold tolerance, drought tolerance, growth rate, disease resistance, heat tolerance, tolerance to extreme osmotic conditions, yield improvement by modification of photosynthesis, modifying seed oil/protein yield and/or content, modification of nitrogen/carbohydrate/ phosphorus use and/or uptake, improved plant growth and development under at least one stress condition;
- FuturaGene PLC recently licensed drought tolerance technology to Bayer CropScience for cotton. The strategy is based on the modification of pathways in stomata opening and closing. The company recently has been granted a patent on the technology by the Chinese patent office. In cooperation with a Chinese company salt tolerant cotton will be developed; and
- Ceres Inc. has filed a patent claiming a multitude of genes involved in drought and heat tolerance (WO 2009/102965). In the same way patent WO 2009/114733 lists sequences of genes that might be helpful in improving tolerance to salt and oxidative stress.

3.2.1 Signal sensing, perception and transduction

The sensors that reside in the cell membrane are part of the initial response to abiotic stress. They transfer the signal via a cascade of mitogen-activated protein (MAP) kinases or calcium/calmodulin-dependent protein kinases further down to the TFs.

- The *Arabidopsis thaliana* plasma membrane histidine kinase (AtHK1) has been suggested to act as an osmosensor that detects water stress. HK2 from maize was listed in 2 maize field trial applications, but no stress related phenotype was mentioned (US applications 07-065-125N and 08-142-108N).
- BRI1, brassinosteroid-insensitive 1 receptor serine/threonine kinase is another sensor that was included in a cotton trial (US application 08-073-110N, *Arabidopsis* gene) in both sense and antisense, together with glutathione reductase that is an important component of the cell's scavenging system for reactive oxygen compounds (ROS) in plants. The desired phenotype is drought and/or heat tolerance.
- OsTPP1 functions as a signalling protein. Expression under the CaMV 35S promoter in rice improves salt and cold tolerance (Ge *et al*., 2008). The authors studied expression patterns in the transgenic rice and found that genes typical for stress responses like for MAP kinases, cold responsive genes *Lip5*, *Lip9* and *DREB1B* and the osmotic responsive gene *OsAsr1* (see below) were up-regulated.
- Genes for calcium dependent protein kinases, calmodulin-binding protein and MAP kinases are mentioned in studies with black nightshade and *N. attenuata* without referring to abiotic stress tolerance (US applications 06-242-01R and 06-242-03R resp.). However, the study subject is most probably biotic stress responses and this is not surprising as these genes signal both types of responses.
- A20/AN1 zinc finger proteins are proteins that are involved in stress response (cold, desiccation, salt, submergence, heavy metals and wounding) but their exact molecular mechanism of action in not yet known (Vij & Tyagi, 2008). It was suggested that these proteins are involved in the stress signalling cascade.
- OsiSAP1 (*O. sativa* subspecies *indica* stress-associated protein), a zinc finger protein from rice was constitutively over-expressed in tobacco leading to cold, salt and drought stress tolerance as assessed at the seedling stage (Mukhopadhyay *et al*., 2004).
- In transgenic tobacco an A20/AN1 zinc-finger ''*AlSAP*'' gene from the halophyte grass *Aeluropus littoralis* improves drought and salt stress (Ben Saad *et al.*, 2010). The authors show that the *AlSAP* gene in *A. littoralis* was induced by salt, drought, cold, heat, ABA and salicylic acid. They also show that the steady state level of transcripts of some stress associated genes encoding proteins involved in anti-oxidative and protection activities are higher in unstressed *AlSAP* tobacco than in WT plants. They propose a role in the phosphorylation cascade that targets proteins directly involved in cellular protection or TFs controlling specific sets of stress-regulated genes.
- The *Arabidopsis* gene for A20-like protein has been included in peanut trials (US notification 09-103-109N) to increase yield, and in cotton (US notifications 09-093-113N and 08-073-110N) to induce drought tolerance. An *Arabidopsis* AN1-like zinc finger protein has been expressed in cotton and trialled under US notification 06-272-103N.

3.2.2 Transcription control & regulatory functions

The regulators that direct the plant's response to stress are the TFs. TFs act as control switches for the coordinated expression of other genes in defined metabolic pathways. Most of these TFs belong to a few large multi-gene families, designated by their common motifs in their nucleic acid sequence. It does not mean that individual members act in the same biological process or at the same time in development. These TFs could regulate various stress-inducible genes cooperatively or separately, and some stress responsive genes may share the same TFs leading to what is called cross-talk.

Figure 9 depicts some of the relationships and pathways that will be discussed further on.

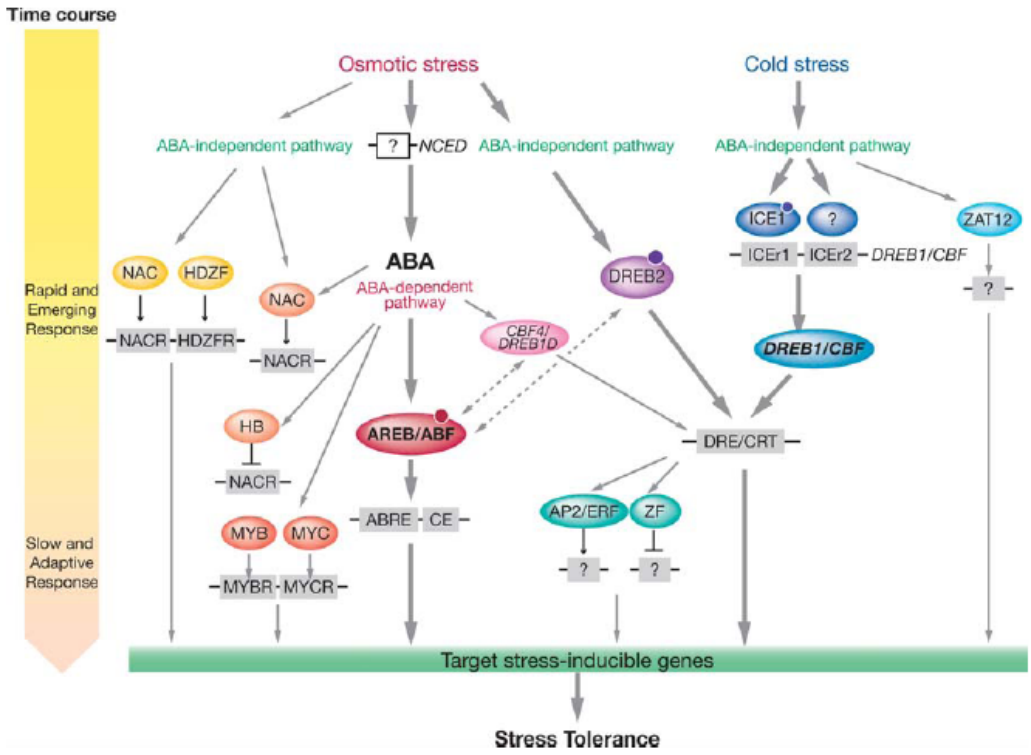

Fig.9 Transcriptional regulatory networks of cis-acting elements and transcription factors involved in osmotic- and cold-stress-responsive gene expression in *Arabidopsis*. (From Yamaguchi-Shinozaki & Shinozaki, 2006; Copyright 2006 Annual Reviews, with permission)

Stress induces a range of physiological and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis and activation of respiration. A cascade of events on the molecular and cellular level takes place. Shinozaki and Yamaguchi-Shinozaki (2007) state that there are at least six signal transduction pathways in drought, high salinity, and cold-stress responses, of which three depend on ABA whereas the other three function independent of ABA.

Saibo and colleagues (2009) discern at least four regulons in the plant response to abiotic stresses. A group of genes controlled by a certain type of TF is known as a regulon. Regulons that can be identified are:

1) the CBF/DREB regulon;
2) the NAC (NAM, ATAF and CUC) and ZF-HD (zinc-finger homeodomain) regulon;
3) the AREB/ABF (ABA-responsive element-binding protein/ ABA-binding factor) regulon; and
4) the MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene) regulon.

ABA induces TFs of the AREB/ABF and MYC/MYB gene family (Saibo *et al.*, 2009). The ABA-responsive element binding factor/ABA-responsive element (ABF/ABRE) family are basic leucine zipper (bZIP) TFs that bind to the ABRE motif and activate ABA-dependent gene expression.

The MYB TFs owe their name to a common highly conserved DNA binding domain that was originally described in the oncogene (*v-myb*) of avian myeloblastosis virus (Klempnauer *et al.*, 1982). Introduced in sugarcane the *Arabidopsis* AtMYB2 protein (myeloblastosis interacting protein 2) is expected to protect against salt stress and dehydration (Australian application DIR070/2006). The transgene was constitutively expressed (maize ubiquitin promoter). Transgenic *Arabidopsis* plants over-expressing AtMYB2 cDNAs have higher sensitivity to ABA (Abe *et al*., 2003) and thereby increase the level of response to ABA that in its turn is increased under drought conditions. The rice *OsMYB4* gene was inserted in Bahia grass and trialled in the US (US applications 06-221-01R and 05-294-01R). Performance Plants patented the use of

MYB-subgroup 14 polynucleotides, *e.g.* MYB68, to enhance a plant's heat tolerance (WO 2009/027824). Examples are given for *Arabidopsis* with both constitutive and inducible promoters. The plants also showed a reduced flower abortion following heat stress.

In the ABA-independent pathways CBF/DREB and ZF-HD families are involved.

The C-repeat-binding factor /dehydration-responsive element binding protein (CBF/DREB) genes are transcription factors that belong to the AP2/EREBP family of DNA binding proteins. CBF proteins recognize and bind to a cold- and drought-responsive DNA regulatory sequence designated as the C-repeat (CRT)/dehydration-responsive element (DRE), which is found in the promoter regions of many cold-inducible genes (Yamaguchi-Shinozaki & Shinozaki, 2006 and references therein). These proteins contain the conserved DNA-binding domain found in the ERF (ethylene-responsive element-binding factor) and AP2 proteins. *Arabidopsis* DREB1 and DREB2 clusters, separate two cellular transduction pathways in low-temperature- and drought-responsive gene expression, respectively, but cross-talk exists. Downstream of these genes several others are activated, such as *STZ*, that functions as a transcriptional co-activator. The *RESPONSIVE TO DEHYDRATION 29A* (*RD29A*), a gene induced by drought, high salinity and cold is another example of a TF induced gene.

The most straightforward way to engineer stress tolerance by these TFs is over-expression using a constitutive promoter. It does lead to the desired stress tolerance, however, a common undesirable side-effect of constitutive over-expression of the *CBF* genes is plant growth retardation, hence stress inducible promoters are used (Yamaguchi-Shinozaki & Shinozaki, 2006).

- The *Arabidopsis* AtDREB1A has been used in soybean (US application 08-081-103N) to induce drought tolerance.
- Orthologues have been isolated in other plant species and used to make stress tolerant GM crops: rice *OsDREB1A* in Bahia grass (US applications 06-221-01R and 05-294-01R) and in sugarcane (Australian application DIR095/2009), *OsDREB1E*, *OsDREB1G*, and *OsDREB2B* in rice (Chen *et al*., 2008); wheat *TaDREB2* and *TaDREB3* in wheat and barley (Australian application DIR 077/2007, Mexico field trial in 2004 by CIMMYT) and *Hordeum spontaneum DREB1A* in turf & forage grass (James *et al*., 2007).
- Other examples are *Arabidopsis* CBF1 in potato (US applications 05-138-25N, 07-081-116N, 07-081-117N, 08-105-103N, 08-105-105N, 09-064-110N and 09-064-106N), CBF3 in *Arabidopsis* (US applications 07-142-102N, 08-065-104N and 09-080-101N), again *Hordeum* CBF in Bahia grass (US applications 05-076-11N, 05-294-01R, 05-364-01R, 06-219-01R, 06-221-01R, 07-197-127R) and *Arabidopsis* CBF in cucumber (US application 05-130-07N).
- ArborGen conducted many field trials in the US on *Eucalyptus* but the genes are not disclosed. Furthermore ArborGen applied for deregulation of a cold tolerant *Eucalyptus* event ARB-FTE1-08 in USA (APHIS 08-366-01p). Although the ERA is not yet available, information about the genetic modification may be retrieved from ERAs of previous field trials, that most probably are about the same gene (APHIS-USDA, 2007, 2009a and 2009b)(US applications 08-014-101RM, 08-011-116R and 06-325-111R). The gene of interest is a CBF gene driven by a cold inducible promoter significantly improving freeze tolerance without negatively impacting other agronomically important traits (*i.e.* stunted growth). In patent application WO 2008/121320A3 ArborGen describes the *A. thaliana CBF2* gene or a *Eucalyptus CBF* homologue operably linked to the stress-related gene *A. thaliana* promoter *RD29A*. Other promoters that are suggested are the ones from *Arabidopsis* or *Eucalyptus* dehydrin (see below).
- HARDY is another AP2/ERF-like transcription factor that is used to improve water use efficiency (WUE). It was isolated from *Arabidopsis*. HARDY over-expression in *Arabidopsis* produces thicker leaves with more chloroplast-bearing mesophyll cells, and in rice, there is an increase in leaf biomass and bundle sheath cells that probably contributes to the enhanced photosynthesis assimilation and efficiency (Karaba *et al.*, 2007). It also increases root biomass under drought stress. The transgenic lines have a reduced transpiration rate indicating that HARDY plays a role in stomatal behaviour. In wild type *Arabidopsis* the HARDY gene is expressed in inflorescence tissue including petals, inflorescence stem,

mature seed, and pollen. The gene is probably involved in the maturation of inflorescence stage processes that require protection of tissue against desiccation (Karaba *et al.*, 2007).

The ethylene response factors SNORKEL1 (SK1) and SNORKEL2 (SK2) allow rice to adapt to deep water (Hattori *et al*., 2009). Both genes have an APETALA2/ethylene response factor (AP2/ERF) domain. In wild-type plants the genes are expressed under deepwater conditions in leaf blade, leaf sheath, and basal parts of the stem, including nodes and internodes. Ethylene that accumulates in deepwater, triggers expression of both genes. Signalling by SK genes may be directly or indirectly connected to GA, which then induces internode elongation. Using the actine promoter from rice the genes were over-expressed. These transgenic plants had elongated internodes even in dry conditions.

The *STRESS-RESPONSIVE NAC1* (*SNAC1*) gene was isolated from an upland rice variety and over-expressed in a lowland rice (Hu *et al*., 2006). *SNAC1* encodes a NAC TF with transactivation activity and is induced by drought, predominantly in guard cells. When compared with the wild type, rice plants constitutively over-expressing SNAC1 showed drought tolerance at anthesis and increased drought and salt tolerance at the vegetative stage. Plants were more sensitive to ABA and closed the stomata more quickly. The plants were not dwarfed as often seen with CBF/DREB TFs.

In Germany potato field trials were performed with a gene for subtilisin like serine protease (SDD1) (EU applications B/DE/04/159 and B/DE/05/167). SDD1 is thought to act extracellularly in the apoplast of stomata precursor cells where it may be involved in the generation of signals responsible for stomata density regulation (von Groll *et al*., 2002). With the CaMV 35S promoter the number of stomata was decreased and plants were highly sensitive for high light intensities in growth rooms. Silencing *SDD1* by RNAi had the opposite effect, namely increasing the number of stomata in the leaves of the plants. Under high light conditions, these plants do not show stress symptoms (*e.g.* leaf rolling), suggesting a higher tolerance to high light/high temperature conditions. Also tuber production was improved. In the field the sense construct plants had a lower tuber yield than the parent plants, but the yield data of RNAi plants did not significantly differ from those of the control plants.

Biogemma trialled maize modified with a construct containing the maize gene for abscisic acid stress ripening induced 1 (ASR1) coupled to the Cassava vein mosaic virus (CsVMV) promoter (EU application B/FR/05/02/02). ASR1 confers Zn^{2+}-dependent, sequence-specific DNA binding activity (Kalifa *et al*., 2004). The Biogemma patent application WO 2001/083756 mentions that *asr1* is induced by ABA but does not describe a possible mode of action in response to drought stress. Shkolnik and Bar-Zvi (2008) report that when tomato ASR1 is over-expressed in *Arabidopsis* it competes with endogenous TF abscisic acid-insensitive 4 (ABI4) on specific promoter DNA sequences. Expression of both ABI4 and ABI4-regulated genes is markedly reduced in transgenic plants. Tobacco plants over-expressing tomato ASR1 demonstrate dramatically increased salt tolerance (Kalifa *et al*., 2004). Plants germinated better in salt conditions and better tolerated salt shock. Several proteins known to be involved in stress response were up-regulated in these plants.

Mendel Biotechnology patent applications WO 2005/047516 and 20090265813 (US Patent) list many TFs among which the WRKY family that is characterized by its 60 amino acid conserved DNA binding domain and a zinc finger domain. Over-expression makes plants more tolerant to low nitrogen conditions and less sensitive to chilling. Also mentioned is the G47 subclade in the AP2 family of TFs that increases tolerance to drought upon over-expression. In Guayule (*Parthenium argentatum* a latex producing *Asteracea*) field trials in the US the *Arabidopsis* G47 type TF is included (US application 08-056-103R). Barley WRKY8 was inserted in Bahia grass (US applications 05-294-01R and 06-221-01R). In transgenic rice the *OsWRKY11* gene under the control of a heat shock promoter promotes heat and drought tolerance (Wu *et al*., 2009). Some events were dwarfed due to a leaking promoter as is seen with constitutive expression of many stress related TFs. Soybean *WRKY* genes were studied in *Arabidopsis* revealing differential roles in abiotic stress as well as in plant development (Zhou *et al*., 2008). WRKY proteins are also involved in pathogen and disease response.

Zinc finger homeodomain (ZF-HD) and basic/helix-loop-helix (bHLH) TFs were also included in the patents from Mendel Biotechnology.

HaHB-4 is a sunflower homeobox-leucine zipper protein (HD-Zip) that in transgenic plants improves drought and salt tolerance using a stress inducible promoter (Argentinean patent application AR039518 A1; US patent application 20070180584). Bioceres S.A. applied for field trials in Argentina with wheat in 2007 and 2008, and in 2008 also with soybean and maize under greenhouse conditions. A research agreement has been announced with Advanta,India to develop drought tolerant sorghum, rice, oilseed rape and cotton. The same gene already showed drought tolerance in *Arabidopsis* (Dezar *et al.*, 2005). But because of the constitutive CaMV 35S promoter the plants were shorter compared to wild type with compact inflorescences. Using the *rd29* or the *Hahb-4* own promoter resolved this problem. In wild type sunflower *Hahb-4* is up-regulated by drought and ABA in roots, stems and leaves.

Another patent was granted to Bioceres S.A. on the use of the *HaHB-10* gene (Argentinean patent application AR053194 A1; US patent application 20070234439). This gene shortens the plant's life cycle and protects against oxidative stress (Rueda *et al*., 2005).

Also, AT-hook TFs are used to improve stress tolerance in crops. US field trial 08-056-103R with Guayule mentions an AT-hook gene to increase drought tolerance. Mendel Biotechnology patented this type of TFs (G1073 clade member) to be used in modifying abiotic stress tolerance (WO/2005/030966).

Monsanto describes a HAP3 transcription factor in combination with water-deficit-inducible promoter to improve the level of drought tolerance (US Patent Application 20080104730).

Monsanto and Mendel Biotechnology together published results on the effect of over-expressing AtNF-YB1 and ZmNF-YB2 in *Arabidopsis* and maize (Nelson *et al*., 2007). The nuclear factor Y (NF-Y) family of TFs are active as heterotrimeric complexes consisting of NF-YA, NF-YB, and NF-YC subunits. Constitutive over-expression lead to increased photosynthetic rates compared to wild type in drought conditions. Transgenic plants had a significant tolerance to drought resulting in increased yield as demonstrated by harvest data from field trials.

Performance Plants filed patent application WO 2004/020642 on the use of farnesyltransferase. Farnesyltransferase is known as a key negative regulator controlling ABA sensitivity in the guard cells. Down-regulation of either the α- or β-subunit of farnesyltransferase enhances the plant's response to ABA and drought tolerance (Wang *et al*., 2005, 2009). Transgenic *Brassica napus* carrying an antisense construct driven by a drought-inducible *RD29A* promoter indeed reduced transpiration. In optimal conditions no yield penalty was shown (Wang *et al*., 2005). Also, the promoter of *Arabidopsis* hydroxypyruvate reductase (*AtHPR1*), which expresses specifically in the shoot and not in non-photosynthetic tissues such as root, gives good results in combination with an RNAi construct in oilseed rape in the field (Wang *et al*., 2009).

Other drought inducible TF genes were inserted in Bahia grass to improve cold and/or drought tolerance, but were not further specified (US application 07-197-127R, barley and rice genes).

3.2.3 Detoxification

Plants coping with environmental stress produce ROS such as O_2, H_2O_2, and OH^- (Chaves & Oliveira, 2004; Umezawa *et al*., 2006; Valliyodan & Nguyen, 2006; Vinocur & Altman, 2005; Wang *et al.* 2003). H_2O_2 acts as a local or systemic signal for leaf stomata closure, leaf acclimation to high irradiance, and the induction of heat shock proteins. H_2O_2 production is also induced by ABA. However, when the production of H_2O_2 exceeds a threshold, programmed cell death might follow (Chaves & Oliveira, 2004 and references therein). Also NO, a reactive nitrogen species, acts as a signalling molecule.

Plants have developed several antioxidation strategies to scavenge the toxic compounds that are formed in stress and that damage membranes and macromolecules. Antioxidants (ROS scavengers) include enzymes such as catalase, superoxide dismutase (SOD), ascorbate

peroxidase (APX) and glutathione reductase, as well as non-enzyme molecules such as ascorbate, glutathione, carotenoids, and anthocyanins (Wang *et al.* 2003).

- Catalases convert hydrogen peroxide molecules to water and oxygen. Genes for catalases have been used to improve stress tolerance in cotton (US notifications 05-066-05N, 05-066-07N, 05-066-08N, 05-066-09N, 05-298-02N, 06-107-102N, 06-107-103N, 06-272-103N, 07-110-102N, maize gene) and tomato (06-139-106N, antisense tomato gene).
- Mn-SOD, Fe-SOD and Cu/Zn-SOD have been used in alfalfa and in tobacco with some success (Wang *et al.* 2003). Mn-SOD from *Nicotiana plumbaginifolia* was field trialled in cotton (US notifications 05-298-02N and 06-272-103N) and in rice (India: strip trials in 2008 and biosafety and bioefficacy trials in 2009).
- Another enzyme, germin-like 1, that has Mn-SOD activity has been expressed in cotton (US application 06-272-103N, 08-073-110N, 09-093-113N, cotton gene).
- Ascorbate peroxidases (or APX1) are enzymes that detoxify H_2O_2 using ascorbate as a substrate. The reaction they catalyse is the transfer of electrons from ascorbate to H_2O_2, producing dehydroascorbate and water as products. Again, cotton has been genetically modified with ascorbate peroxidase genes to improve stress tolerance (US applications 05-066-05N, 05-066-07N, 05-066-08N, 05-066-09N, 05-298-02N, 06-107-102N, 06-107-103N, 06-272-103N, 07-110-102N, 08-109-101N, 09-093-113N, pea gene).
- In several of these field trial applications also glutathione reductase is mentioned (US applications 05-066-05N, 05-066-07N, 05-066-08N, 05-066-09N, 05-298-02N, 06-107-102N, 06-107-103N, 06-272-103N, 08-073-110N, *Arabidopsis* gene).

Reduced glutathione (GHS) acts as a part of the ascorbate-glutathione cycle to scavenge H_2O_2 (Srivalli & Khanna-Chopra, 2008). It is the reducing agent that recycles ascorbate from its oxidized form (dehydroascorbate) to its reduced form by the enzyme dehydroascorbate reductase. The regeneration from oxidized glutathione reduced glutathione is catalyzed by glutathione reductase. GHS also plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in the reduced state. It can also function directly as a free radical scavenger by reacting with superoxide, singlet oxygen, and hydroxyl radicals. GHS protects proteins against denaturation caused by the oxidation of protein thiol groups under stress. In addition, GSH is a substrate for glutathione peroxidase (GPX) and glutathione-S-transferases (GST), which are also involved in the removal of ROS (Srivalli & Khanna-Chopra, 2008). Also these enzymes have been studied to increase stress tolerance (Roxas *et al*. 1997).

Alternative oxidase (AOX) provides an alternative route for electrons passing through the electron transport chain to reduce oxygen. The expression of the alternative oxidase gene AOX is influenced by stresses such as cold, ROS and infection by pathogens, as well as other factors that reduce electron flow through the cytochrome pathway of respiration. A *N. tabacum* AOX1 was introduced in cotton for cold tolerance (US notification 07-110-102N), and the maize AOX3 was over-expressed in maize (US notification 05-084-04N). The enzymes were also included in research trials by the Max Planck Institute for Chemical Ecology in *N. attenuata* and black nightshade (US applications 06-003-08N, 06-242-01R and 06-242-03R, *Solanum nigrum* and *N. attenuata* genes).

Cytochrome P450 enzymes represent a large family that are usually part of an electron transfer chain. They are mono-oxygenases that use a lot of substrates to transfer one oxygen atom from O_2 to, while the other is reduced to water. US applications 09-093-118N (*Streptomyces griseolus* gene) for cotton and 08-077-104R (*Oryctolagus cuniculus* gene) for hybrid poplar exploit such an enzyme.

Poly(ADP-ribose) polymerase (PARP) is a protein involved in a number of cellular processes involving mainly DNA repair and programmed cell death. PARP catalyzes the post-translational modification of proteins by adding successively molecules of ADP-ribose, obtained from the conversion of nicotineamide dinucleotide (NAD), to form multi-branched polymers containing up to 200 ADP-ribose residues. It is suggested that this post-translational modification is involved in metabolism of nucleic acids, and DNA repair. NAD^+ depletion in turn results in ATP depletion, because NAD^+ resynthesis requires at least (depending on the biosynthesis pathway) three molecules of ATP per molecule of NAD^+. Furthermore, NAD^+ depletion blocks glyceraldehyde-3-

phosphate dehydrogenase activity, which is required to resynthesize ATP during glycolysis. Finally, NAD^+ is a key carrier of electrons needed to generate ATP via electron transport and oxidative phosphorylation. Plants activate this gene as soon as DNA molecules break down as a direct or indirect result of stress. Because of this up-regulation the plant looses a lot of energy. By suppressing the gene's activity using RNAi hardly any energy is wasted and still plants do better resist abiotic stress. Field trials have been started in Puerto Rico, US with maize (US notifications 03-290-03N and 04-336-03N). The invention was patented by Bayer Bioscience (WO 2004/090140) and is applied in oilseed rape in particular.

Another way to solve the problem of NAD^+ depletion is the resynthesis of NAD^+ from its degradation product in the so-called NAD salvage pathway. In a first step, nicotinamide is deamidated to nicotinic acid by a nicotinamidase. The nicotinic acid is transferred to 5-phosphoribosyl-1-pyrophosphate by the enzyme nicotinate phosphoribosyl transferase to yield nicotinic acid mononucleotide. This molecule is further converted to NAD by NAD^+ pyrophosphorylase and NAD synthetase (patent applications WO 2006/032469 and WO 2007/107326). The invention then describes that introducing one or more yeast or fungal genes selected from the salvage synthesis pathway increases the stress resistance of a plant. Plants also exhibited a significantly reduced level of ROS. An example is given for *Arabidopsis* and oilseed rape.

Some other examples are the aldehyde dehydrogenase AtALDH3 gene in *Arabidopsis* (Sunkar *et al.*, 2003) and glyoxalase I (*gly I*) and glyoxalase II (*gly II*) required for glutathione-based detoxification of methylglyoxal which is formed primarily as a byproduct of carbohydrate and lipid metabolism (Singla-Pareek *et al*., 2003). Aldehyde dehydrogenase catalyzes the oxidation of toxic aldehydes, which accumulate as a result of side reactions of ROS with lipids and proteins. Transgenic *Arabidopsis* show improved tolerance when exposed to dehydration, NaCl, heavy metals and H_2O_2. *Gly I* has been shown to be up-regulated in tomato in response to salt and osmotic stress and to phytohormonal stimuli. In *Arabidopsis* it is induced in response to drought and cold stresses. Over-expression of both genes also resulted in salinity-tolerant tobacco plants and rice (Singla-Pareek *et al*., 2008). The over-expression of glyoxalases could enhance the level of reduced glutathione that presumably helps to detoxify ROS.

In the Australian field trial applications DIR 067/2006 and DIR 083/2007 GM cotton lines contain an *AHb1* gene derived from *Arabidopsis* that encodes a class 1 non symbiotic phytohaemoglobin protein (nsHb) expected to provide tolerance to waterlogging stress. The exact mechanism of action of plant nsHbs is unknown. The proteins act as oxygen carriers and studies have shown that over-expression of *AHb1* in GM *Arabidopsis* leads to higher survival rates and increased shoot and root weight after hypoxia than in non-GM plants. In wild type *Arabidopsis, AHb1* showed the highest developmental expression during germination. The over-expression of *AHb1* in GM *Arabidopsis* also leads to enhanced early growth rates under normal growth conditions due to increased size of roots and shoots. Igamberdiev and colleagues (2005) suggest a role in NO scavenging and homeostasis. NO is known to cause toxic effects at high concentrations and has been implicated in programmed cell death. Oxygen deficiency leads to a decline in mitochondrial respiration, triggering an increase in NADH and a drop in ATP levels. This results in class 1 *nsHb* gene expression and activation of NR, leading to production of NO. This NO, in combination with the oxygenated form of newly synthesized nsHb, leads to the oxidation and oxygenation of NO, resulting in restoration of cell redox and energy status. NO also induces ethylene production, activates MAP kinases and guanylate cyclase, resulting in aerenchyma formation.

In both field trials the *AHb1* is controlled by the constitutive subterranean clover stunt virus (S4S4) promoter. In DIR 083/2007 also pyruvate decarboxilase (PDC) and alcoholdehydrogenase (ADH) is added. PDC that catalyses the first step of the alcoholic fermentation pathway using pyruvate as substrate. ADH catalyses the second step leading to ethanol biosynthesis. Both genes are driven by the CaMV 35S promoter. The ethanol biosynthesis pathway (fermentation) is an alternative for energy production under low oxygen conditions.

CropDesign filed a patent on plant haemoglobins (WO 2004/087755). Examples are given of a sugarbeet class 2 *nsHb* introduced in *Arabidopsis* (CaMV 35S promoter) or rice (*GOS2* promoter) to improve salt stress tolerance. These class 2 proteins are normally expressed during embryogenesis and seed maturation, around openings (*e.g.* stomata) or at branch points. They respond to cytokinins and are induced in *Arabidopsis* upon cold, osmotic and saline stress.

Early Light Induced Proteins (ELIP) are induced upon several types of environmental stress, in particular photo-oxidative stress. ELIPs are transiently expressed and are transported to the thylakoid membranes containing the chlorophyll molecules. ELIPs presumably protect the photosystem II. Maize transformed with a Cassava vein mosaic virus (CsVMV) promoter - *ELIP*1 or *ELIP2* construct is tolerant to photo-oxidative stress. Biogemma patented this invention (patent application WO 2003/074713).

3.2.4 Chaperone functions

Maintaining proteins in their functional conformations and preventing aggregation of non-native proteins under stress conditions are particularly important for cell survival under stress. Molecular chaperones function in the stabilization of proteins and membranes, and in assisting protein refolding under stress conditions (Wang *et al*., 2003). Heat-shock proteins (HSPs) and late embryogenesis abundant (LEA)-type proteins are the two major types.

HSPs function during both normal cell growth and stress conditions. They have been reported to be involved in protein import and translocation and in facilitating the proteolytic degradation of unstable proteins (Wang *et al*., 2004), Small HSPs are involved in many developmental processes, such as embryo development, seed germination, somatic embryogenesis, pollen development, and fruit maturation Wang *et al*., 2003).

Various studies have shown that small HSPs in plants are not only expressed in response to heat shock but also under water, salt, and oxidative stress, and at low temperature (references in Wang *et al*., 2003). They even might act as antioxidant. The *Arabidopsis HSP101* gene was introduced in rice (Katiyar-Agarwal *et al*., 2003) and in cotton (US notification 07-082-104N) to increase heat tolerance. Heat shock proteins were also tested in maize (US notification 05-084-04N, maize gene) and a heat shock binding factor 1 in cotton (US notification 06-272-103N, *Arabidopsis* gene).

The mitochondrial small heat shock protein AB017134 from tomato was over-expressed in tomato (US notification 06-139-106N) again to improve the plant's tolerance to heat. In tobacco the tomato gene already induced thermotolerance (Sanmiya *et al.*, 2004). Small heat shock proteins bind to thermally denatured proteins at their surface to maintain a folding-competent state. The mitochondrial small heat shock proteins protect the mitochondria from breaking down and have a pivotal role in the response to heat shock in tobacco plants.

Also the promoters of HSPs may be useful in engineering stress tolerant crops. In the EU application B/ES/09/57 a gene promoting tuberization (not further disclosed) under control of a heat shock promoter from soybean is tested in potato.

LEA proteins are expressed at different stages of late embryogenesis in higher plant seed embryos and under conditions of dehydration stress. They are very heat stable and their related gene expression is transcriptionally regulated and responsive to ABA (references in Wang *et al*., 2003).

OsLEA3a is a rice gene induced by drought, salt and ABA, but not by cold stress (Xiao *et al*., 2007). Using the gene's own promoter or a CaMV 35S promoter increased yield in a transgenic, drought sensitive rice variety without yield penalty in normal conditions. CropDesign showed that also under normal growing conditions OsLEA3a when over-expressed with the rice GOS2 promoter increases rice yield (patent application WO 2007/031581).

A wheat group 2 LEA protein, also known as dehydrin (DHN-5) was used to transform *Arabidopsis* (Brini *et al*., 2007). In wild-type *T. durum* plants the *dhn-5* gene is induced during

embryogenesis and also by ABA and salt stress in vegetative tissues. Transgenic *Arabidopsis* were more resistant to osmotic stress by mannitol or to salt and drought stress when grown in soil. Also, proline levels were increased (see below). Several *dhn* genes from *Hordeum spontaneum* were tried in Bahia grass (US applications 05-294-01R, 06-221-01R and 07-197-127R). The use of *dhn1* to obtain abiotic stress tolerance was patented (US 2008/0196126). As an example barley was transformed with the *dhn1* gene from *H. vulgare* ssp. *spontaneum*.
Dehydrins are activated by CBF/DREB TFs (Valliyodan and Nguyen, 2006).

Expression of HVA1, a group 3 LEA protein from barley conferred tolerance to soil water-deficit and salt-stress in transgenic rice plants (Xu *et al.*, 1996; Babu *et al.,* 2004), and improved biomass productivity and water use efficiency under water-deficit conditions in transgenic wheat (Sivamani *et al.*, 2000). Oraby and colleagues (2005) demonstrated that the *HVA1* gene conferred salt tolerance to oat in greenhouse conditions. Plants recovered better from the stress treatment. Heading, a development that plants try to bring forward when stressed, was later than in wild-type plants.

RNA chaperones are ubiquitous and abundant proteins found in all living organisms and viruses. Under stress conditions RNA tends to misfold and then becomes unable to perform its normal function (Castiglioni *et al*., 2008 and references therein). Chaperone proteins can resolve these structures. Bacterial cold shock proteins (CSPs) can account for as much as 10% of the newly synthesized protein of cold-shocked cells. CSPA from *E. coli* binds to RNA and, when necessary, converts double-stranded RNA into single-stranded RNA. Similarly, the endogenous function of CSPs in plants relies on RNA binding/chaperone activity. These proteins contain a nucleic acid binding domain. They have also been demonstrated to play a role in RNA metabolism, protein translation, and regulation of gene expression.

Transgenic *Arabidopsis* plants either containing the *E. coli cspA* gene or a *Bacillus subtilis cspB* gene demonstrated improved growth under low temperature conditions (Castiglioni *et al*., 2008). In rice both genes improved stress tolerance for a number of abiotic stresses, including cold, heat, and water deficits. Plants recovered better after stress as measured by plant height. Transgenic expression of *cspB* in maize plants contributes to improved vegetative performance. In drought periods during vegetative growth the best performing events demonstrated growth rate increases of 12% and 24% (field trial). Chlorophyll content and photosynthetic rates were increased. But in maize, floral transition and reproductive development stages are the most sensitive to the water deficit stress conditions in terms of yield impact. When water was limited during the late vegetative phase of development, again, the modified plants performed better than the control plants. In optimal conditions the *csp*A or *csp*B plants were not different from non-transgenic plants both in greenhouse and field environments indicating that the extra genes did not cause a yield penalty. One *cspB* event was chosen to further test drought tolerance during the generative phase. Under controlled water-deficit conditions this event was tested in three different hybrid backgrounds. The yield of control plants was reduced to 50% compared to well-watered conditions. Yield benefits in these experiments ranged from 11% to as much at 21% in this event.

After trialling in the US, Canada, South Africa, Argentina and Chile; event MON87460 expressing the *cspB* from *Bacillus subtilis* has been notified for commercial use in the US, EU, Canada, Australia, New Zealand and the Philippines. The gene is constitutively expressed through the rice actin 1 promoter.

Spermidine and spermine and their precursor putrescine are polyamines involved in the regulation of various cellular and molecular processes. They associate with DNA, RNA and proteins and membranes, but they also function as osmolytes. The exact mechanism of their function in stress response is not clear yet.

Over-expression of arginine decarboxylase (ADC), ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC), enzymes in the biosynthesizing pathway, induce a significant increment in putrescine levels and a small increase in spermidine and spermine levels (Vinocur & Altman, 2005 and references therein). Transgenic rice plants expressing the *Datura*

stramonium adc gene (Capell *et al*., 2004) using the maize ubiquitin promoter elevate drought tolerance. The hypothesis is that under stress constitutive expression augments the putrescine pool to levels that extend beyond the critical threshold required to initiate the conversion of excess putrescine to spermidine and spermine. Putrescine levels in wild-type plant do increase under stress but insufficiently to trigger the conversion of putrescine into spermidine and spermine.

Spermidine synthase cDNA from *Cucurbita ficifolia* was introduced in *Arabidopsis thaliana* under the control of the cauliflower mosaic virus 35S promoter (Kasukabe *et al*., 2004). The spermidine content in leaves was significantly increased together with enhanced tolerance to various stresses including chilling, freezing, salinity, hyperosmosis and drought. Stress-responsive transcription factors such as DREB and stress-protective proteins like RD29A were more abundantly transcribed in the transgenics than in the wild type under chilling stress.

Spermidine synthase SPE3 and S-adenosyl methionine decarboxylase both from *Saccharomyces cerevisiae* have been used in tomato field trials (US applications 07-115-101N and 06-139-106N). However, it is not certain whether these genes are intended for stress tolerance or for improving nutrient quality as also the lycopene content might be increased (Mehta *et al*., 2002). It was also suggested that polyamines act as anti-senescence and anti-ripening regulator by inhibiting ethylene biosynthesis and conversely that ethylene inhibits polyamine biosynthesis (Mehta *et al*., 2002 and references therein). As a result of the over-expression of S-adenosyl methionine decarboxylase in ripening fruits the vine life is extended. Indeed, as antioxidants, polyamines may protect against oxidative degradation and membrane damage resulting in a longer ripening phase.

3.2.5 Osmoprotection

Osmolytes are organic compounds affecting osmosis and playing an important role in maintaining cell volume and fluid balance. A broad range of compounds has been identified as playing a protective role on membranes and macromolecules. They comprise proline, glutamate, glycine-betaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose, and oligosaccharides. All these compounds enable the proteins to maintain their hydration state (Chaves & Oliveira, 2004). The molecules, compatible solutes, are non-toxic and do not interfere with normal metabolism. They accumulate predominantly in the cytoplasm at high concentrations under osmotic stress (Bartels & Sunkar, 2005).

3.2.5.1 Proline biosynthesis

The enzyme ornithine aminotransferase converts ornithine to pyrroline-5-carboxylate. Alternatively pyrroline-5-carboxylate synthase catalyzes the conversion of glutamate to pyrroline-5-carboxylate. Next pyrroline-5-carboxylate reductase reduces pyrroline-5-carboxylate to proline. Proline may be metabolized to glutamate with the aid of proline dehydrogenase (Bartels & Sunkar, 2005; Wang, 2003). Modifying the expression of several of these enzymes may increase the level of proline in the plant cell.

The ornithine aminotransferase gene (*δ-oat*) from *Arabidopsis* was transferred to wheat to improve its salt tolerance (Australian application DIR 053/2004). For hydroponically grown plants under salt-stress conditions (150 mM NaCl) the free proline concentration in extracts from the GM wheat plants was two (per g fresh weight) to three (per mg protein) times that in non-GM wheat. GM plants had a greater than two-fold increase in tiller number, seed number and seed weight in comparison to the non-GM wheat under saline hydroponic conditions. Except for a slightly smaller stature no difference was detected in the absence of salt stress.

Hong and colleagues (2000) over-expressed a pyrroline-5-carboxylate synthase gene in tobacco. The gene encoded a mutated form of the enzyme whose feedback inhibition by proline was removed. The transgenic plants better withstood salt stress.

Another strategy is preventing the degradation of proline by antisensing the proline dehydrogenase gene (Nanjo *et al*., 1999). Modified *Arabidopsis* plants were more tolerant to freezing and saline conditions.

3.2.5.2 Glycine-betaïne

In a two-step oxidation choline is transformed to produce glycine-betaine via an unstable, toxic glycine-betaine aldehyde intermediate. The first step is in several higher plants catalyzed by choline monooxygenase, the second by betaine aldehyde dehydrogenase both located in the chloroplast (Bartels & Sunkar, 2005; Sakamoto & Murata, 2000 & 2002). In microorganisms like *E. coli* betaine is synthesized by choline dehydrogenase catalyzing both steps. In others like *Arthrobacter globiformis* betaine is synthesized in one step by choline oxidase. Many important crops such as rice and potato are betaine-deficient. Some plants like spinach and sugar beet are natural accumulators of glycine-betaine (Sakamoto & Murata, 2000 & 2002).
The betaine aldehyde decarboxylase gene from *Suaeda liaotungensis*, a halophytic plant, has been used to improve tolerance to salinity in transgenic tobacco (Li *et al.*, 2003) and maize (Wu *et al.*, 2008).

The choline oxidase gene (*codA*) from *Arthrobacter globiformis* made rice plants recover from salt stress (Mohanty et al., 2002). In persimmon the same choline oxidase gene was introduced to enhance cold or drought tolerance (US applications 99-048-25N and 08-250-101R).

Monsanto patent WO 2004/101741 describes a gene sequence designated as *GB1* (glycine-betaine 1) to enhance the level of the osmolyte in soybean, cotton, canola or maize. The plants exhibit increased tolerance to water-deficit, cold or freezing growing conditions or increased yield.

3.2.5.3 Mannitol

Mannitol is normally synthesized in numerous plant species, but not in wheat. In celery (*Apium graveolens*), mannitol is synthesized in equal proportion to sucrose. Mannitol accumulation increases when celery plants are exposed to low water potential and accumulation is regulated by inhibition of competing pathways and decreased mannitol consumption and catabolism (Abebe *et al.*, 2003 and references therein). The mannitol-1-phosphatase dehydrogenase gene (*mtlD*) from *E. coli* was introduced in wheat and assayed in field trials in the US (Abebe *et al.*, 2003; US application 07-029-102N). The enzyme catalyzes the reversible conversion of fructose-6-phosphate to mannitol-1-phosphate. In transgenic plants, mannitol-1-phosphate is converted to mannitol via non-specific phosphatases. The growth of transgenic wheat under water stress and salinity was improved both at the callus and whole-plant level. However the amount of mannitol accumulated in response to stress was small. It was suggested that the beneficial effect of mannitol resulted from protective mechanisms other than osmotic adjustment, *e.g.* through scavenging of hydroxyl radicals (OH^-) and stabilization of macromolecular structures. In tobacco, mannitol protects thioredoxin, ferredoxin, and glutathione and the thiol-regulated enzyme phosphoribulokinase from the effects of OH^- (Shen *et al*., 1997).

The same research group applied for field trials with wheat in 2005 and 2006 (US applications 06-018-01N and 05-026-25N, *E. coli* gene). Here the mannitol dehydrogenase which oxidizes mannitol to mannose was the gene of interest to improve drought tolerance.

3.2.5.4 Trehalose

A yeast *TPS1* gene encoding the enzyme trehalose-6-phosphate synthase was introduced in potato to improve drought tolerance. The promoter region of the drought-inducible gene *DS2* derived from the potato was used (B/HU/07/06). This promoter is active in leaves and only induced by dehydration, not by cold, heat, salt or oxidative stress. Research published in 2008 reports that in contrast to the expected drought-induced expression, only a very low constitutive *TPS1* expression was detected in the transgenic lines, probably due to chromosomal position effects (Stiller *et al*., 2008). The observed expression pattern, however, was sufficient to alter the drought response of plants: delayed wilting, prolonged CO_2 assimilation rate and net photosynthesis rate.

EcTPSP, a fusion enzyme of a trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, both from *E. coli,* was used in sugarcane to increase trehalose production with the aim of increasing drought tolerance (Australian application DIR070/2006).

In patent application WO 2006/060376 a method is presented to solve the problem of low carbon assimilation due to closed stomata. By down-regulating the trehalose-6-phosphate phosphatase activity carbon is diverted from the trehalose pathway to be redirected to sucrose or starch synthesis and thus to the developing ovules/embryos resulting in stabilized yield in growing environments that are subject to periodic stress. Down-regulation is accomplished by RNAi technology using the promoter from a *Rab-17* gene. The maize *Rab-17* promoter is drought-inducible in vegetative tissue and developmentally expressed in maturing kernels.

3.2.5.5 Sorbitol

Sorbitol-6-phosphate dehydrogenase (S6PDH) is an enzyme involved in sorbitol biosynthesis. The *S6PDH* gene isolated from apple was introduced in persimmon (US notification 08-250-101R) to induce drought tolerance. The gene is also known to confer salt tolerance in persimmon plants (Deguchi *et al*., 2004). A side-effect is the dwarfed phenotype.

Applications were also submitted for apple modified with *MdS6PDH* (US applications 06-121-103N, 07-177-105N and 08-235-102R). However, it is not clear whether any stress tolerance was envisioned.

Again, in sugar cane this *MdS6PDH* gene was trialled to improve drought tolerance (Australian application DIR070/2006). In greenhouse experiments necrosis is observed on the leaves. The effect is more intense in plants producing larger amounts of sorbitol. Also, the plants remain about 30% shorter.

3.2.6 Proton pumps, antiporters and ion transporters

Salt makes it harder for roots to extract water from the soil and, when taken up, high concentrations of salts within the plant can be toxic. As a result plants cease growth or even die. The most abundant salt in the soil is NaCl. High salt concentrations inhibit the activities of most enzymes. Moreover, Na^+ is competing with K^+, thus disrupting K^+ homeostasis (Bartels & Sunkar, 2005). The cytosolic enzymes in halophyte plants are as sensitive to salt as in other plants, but halophyte plants are able to exclude Na^+ and Cl^- while water is taken up. In other plants these ions, that passively enter the root hair cells, pass the root cells and are transported throughout the plant in the transpiration stream. For most species, Na^+ appears to reach a toxic concentration before Cl^- does, and so most studies have concentrated on Na^+ exclusion and the control of Na^+ transport within the plant (Munns & Tester, 2008). Excluding Na^+ from the leaves can be done in two ways: either Na^+ is prevented from being transported or it is stored in the vacuole (compartmentation).

Most of the Na^+ that enters the root will be pumped out again by Na^+/H^+ antiporters (Aspe & Blumwald, 2007; Munns & Tester, 2008). These Na^+ efflux proteins exchange Na^+ for H^+ requiring energy. One of the genes encoding such protein is the *Arabidopsis SOS1* (salt overly sensitive 1).

The Na^+ that is not exported is pumped to the vacuole or transported to the shoot via the xylem. Compartmentation of Na^+ into the vacuolar lumen against its concentration gradient must be driven, either directly or indirectly, by energy-dependent mechanisms. 'Uphill' transport of Na^+ into the vacuolar lumen is mediated by the operation of tonoplast Na^+/H^+ antiporter, energized by the co-ordinate action of proton pumps, the vacuolar ATPase (V-ATPase) and the vacuolar-pyrophosphatase (AVP) (Aspe & Blumwald, 2007; Bartels & Sunkar, 2005). Tonoplast Na^+/H^+ antiporters are for example those belonging to the Na^+/H^+ exchanger (NHX) family in *Arabidopsis* (Darley *et al*., 2000). Protons are used as coupling ions for ion transport systems, and the proton gradient, generated by proton pumps in the membrane systems, is the driving force for Na^+ transport across membranes. The sequestration of ions such as Na^+ could increase the osmotic

pressure of the plant and therefore the ability to retain water and at the same time reduce the toxic effects of this cation.

Some members of the HKT gene family have a role in retrieval of Na^+ from the xylem before it reaches the shoot, such as the only *Arabidopsis* gene of the family *AtHKT1;1* (Munns & Tester, 2008). AtHKT1;1 functions as a Na^+-selective uniporter with some role in K^+ transport. This plasma membrane protein is expressed in stelar cells in the root and within vascular tissues in leaves (Møller *et al*., 2009). In rice 9 HKT members are known but their function has not always been assigned. OsHKT2;1 also plays a role in Na^+ intake from the soil. In salt tolerant varieties the expression of this gene is down-regulated (Aspe & Blumwald, 2007).

Once in the leaf Na^+ may be directed to the epidermal cells as in barley or to the cell vacuole. AtNHX1 and AtAVP1 may be involved in the latter. If Na^+ and Cl^- are sequestered in the vacuole of a cell, organic solutes that are compatible with metabolic activity even at high concentrations must accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole. These solutes are sucrose, proline, glycine-betaine and others. Their synthesis requires energy and sacrifices resources that normally would go to seed or fruit production for example.

The *Arabidopsis* SOS1 strategy has been studied in *Arabidopsis* (US applications 08-065-104N and 09-080-101N) and trialled in tomato (US application 06-167-102N).

FuturaGene has invested in genes involved in the SOS pathway. *SOS1* is induced by the action of SOS2/SOS3 complex phosphorylating and subsequently activating *SOS1*. *SOS2* encodes a serine/threonine protein kinase regulated by SOS3. The *SOS3* gene encodes a Ca^{2+} sensor protein capable of sensing the cytosolic Ca^{2+} signal elicited by salt stress (Aspe & Blumwald, 2007; Bartels & Sunkar, 2005).

The *NHX1* gene from *Arabidopsis* was introduced in peanut (US application 09-103-109N), in cotton (US applications 06-319-103N and 09-093-113N), in rice with or without the rice equivalent (US applications 07-071-103N, 07-110-101N, 08-029-107N, 08-086-104N and 09-064-104N) and in *Arabidopsis* (US application 07-142-102N). Many of these trials were conducted by Arcadia Biosciences.

He (2005) describes the modification of cotton with *AtNHX1* under the control of the CaMV 35S promoter. The GM cotton plants withstood salt conditions better than control plants not only in the greenhouse, but also in the field (US applications 04-145-04N and 05-066-06N). The plants were taller, had a bigger root mass, displayed a higher photosynthetic rate and a higher nitrate reductase activity than wild-type control plants under salt conditions. The transgenic lines produced more bolls and higher fibre yield with an average increase of 25% per line. The author suggests that the salinity tolerance is however limited by the capacity of the proton pumps. To further increase salt tolerance in plants, the simultaneous over-expression of both vacuolar H^+ pump and vacuolar Na^+/H^+ antiporter will probably be required.

Not further identified Na^+/H^+ exchangers from *Arabidopsis* were used in rice (US applications 05-088-03N and 06-082-08N) and in tomato (US application 05-152-02N).

Patent application WO 2002/016423 describes Na^+/H^+-exchangers (PpNHXl and PpNHX2) from *Physcomitrella patens*, a halophyte. The idea is that antiporters from salt-tolerant plants have functionally more effective sodium transport systems compared to salt-sensitive plants.

Møller and colleagues (2009) studied Na^+ transport processes. They modified *Arabidopsis* with the *AtHKT1;1* gene that was specifically over-expressed in the root stele. As a result Na^+ levels significantly decreased in the shoot, increased in the root and plants were more tolerant to salinity. Plants where the gene was constitutively over-expressed (CaMV 35S promoter) were stunted and displayed chlorosis. The amount of Na^+ in their leaves was 3.6-fold higher on average compared with controls.

CropDesign patented the use of a HKT1 transporter protein (WO 2006/045829). They claim that over-expression of HKT1 results in improved seed yield even in non-stress conditions. An example is given for constitutive expression of an *Arabidopsis HKT* gene in rice.

In cotton an *Arabidopsis* gene for a metal ion/hydrogen ion antiporter was inserted (US application 05-038-01N) and one for a proton transporter (US application 06-080-05N).

Saccharomyces cerevisiae cation transport systems, such as HAL1 and HAL3, are involved in the regulation of K^+ and Na^+ transport, respectively. The proteins decrease intracellular Na^+ levels but retain K^+. Transgenic melon and tomato expressing HAL1 showed some salt tolerance (Wang *et al*., 2003 and references therein).

Transgenic *Arabidopsis* plants over-expressing the vacuolar H^+-pyrophosphatase AVP1 accumulate more Na^+ (in the vacuole) and K^+ in their leaf tissue (Gaxiola *et al.*, 2001). Greater solute accumulation in leaves was identified as one basis for altered response to water deficit. The gene was further studied in tomato where over-expression resulted in increased root biomass (Park *et al*., 2005). It was hypothesized that AVP1 plays an important role in organ development through facilitating the auxin fluxes that regulate organogenesis. The invention was patented (WO 2009/020528). Also, in this patent it is postulated that the uptake of inorganic phosphate (Pi) is enhanced due to the increased H^+ extrusion in the rhizosphere enhanced plasma membrane H^+-ATPase resulting in increased displacement of Pi from insoluble soil complexes.

The same *Arabidopsis* gene has been used to enhance drought or salt stress tolerance in sweet potato (US application 09-159-102N), peanut (US notification 09-103-109N), cotton (US notifications 07-093-104N, 07-137-102N, 08-073-110N and 09-093-113N), creeping bent grass (US application 08-273-101R), *Festuca arundinacea* (US applications 05-312-01R and 06-201-01R), poplar (US application 05-192-14N) and tomato (US application 08-067-110N).

Aquaporin proteins facilitate osmosis by forming water-specific pores as an alternative to water diffusion through the lipid bilayer, thus increasing the water permeability of the membrane (Bartels & Sunkar, 2005 and references therein). Aquaporins are members of a large super-family of membrane spanning proteins, the major intrinsic proteins (MIPs). In plants, aquaporins localized in the tonoplast are called tonoplast intrinsic proteins (TIPs), while those in the plasma membrane are PIPs. Up-regulation of aquaporins, induced by drought or salt may facilitate water flux. However, also down-regulation is observed that may allow cellular water conservation (Bartels & Sunkar, 2005 and references therein). Aquaporins can also transport small neutral molecules like CO_2, H_2O_2, boron or silicon. A high H_2O_2 transport capacity was determined for AtTIP1;1 and AtTIP1;2 pointing at a role in stress signalling (Maurel, 2007). Maize has been modified with a maize TIP1 gene (US application 08-142-108N).

3.2.7 Chemical stress

Addressing abiotic chemical stress tolerance should be distinguished from bioremediation, because the intended use of these differs, and this should factor into the risk assessment. Abiotic stress tolerance is introduced into plants so that they can be grown on land where their productivity is otherwise limited because of the stressor -in this case a chemical compound- that is present there. The intention of engineering plants for bioremediation is not to improve the productivity of the plants in stressed conditions, but to facilitate the removal of a contaminant from the environment. Bioremediation is excluded from this report.

Crop yields are greatly affected by aluminium toxicity in acid soils that cover nearly 40% of the arable land, particularly in tropical and subtropical countries (Vasil, 2007). In the prevalent acidic environment the ionic forms of aluminium that are solubilized into the soil solution are highly toxic to root growth or function resulting in significant yield losses. Transgenic plants over-expressing citrate synthase or malate dehydrogenase genes have been shown to enhance aluminium tolerance (Vasil, 2007 and references therein). The manipulation of organic acid biosynthesis leads to increased secretion of organic acids from roots, and the increased Al tolerance is attributed to the ability of organic acids to chelate and detoxify Al^{3+} (Delhaize *et al*., 2004).

However, the transport of these molecules to the soil appears to be a rate-limiting step. *ALTM1* is a gene encoding an Al-activated anion channel that is permeable to malate and is isolated from wheat. It was introduced into barley to obtain a high level of tolerance to aluminium (Delhaize *et al*., 2004). The native promoter allows expression only in the root tip.

Rutgers University used the citrate synthase gene from *Pseudomonas aeruginosa* to introduce in creeping bent grass (*Agrostis stolonifera*) (US applications 98-103-22N and 99-103-07N).

The Australian field trial application DIR077/2007 describes barley plants to which a boron efflux transporter gene *bot1* with its own promoter is added for boron tolerance. The gene was isolated from an Algerian landrace of barley, which is tolerant to high levels of boron in the soil. Cultivated barley also encodes a copy of this gene, but with several differences in the coding and protein sequences. The landrace has approximately four times more copies of the gene and approximately 160-180 times greater steady-state RNA levels in the leaf blades and root tissue than the intolerant barley cultivar.

On the other hand, in calcareous soils iron complexes although abundantly present are unavailable to the plant. Ferric hydroxide complexes are insoluble in aerobic environments at neutral or basic pH (Connolly *et al*., 2003, Vasconcelos *et al*., 2006). Iron is an essential element as electron acceptor and donor in photosynthesis and respiration and acts as a cofactor in chlorophyll biosynthesis. Plants may mobilize iron from the soil acidifying the rhizosphere by releasing protons. Fe(III) is then reduced by ferric chelate reductase to Fe(II) that is subsequently taken up by ferrous iron transporter into the root epidermis cells. The reductase activity was found to be the rate limiting step (Connolly *et al*., 2003). Soybean was transformed with a CaMV *35S – FRO2* gene construct (Vasconcelos *et al*., 2006). *FRO2* is the *Arabidopsis* ferric chelate reductase gene. Transformants grown in hydroponics with Fe(III)-DTPA as the sole source of iron showed reduced chlorosis compared to the control plants. The plants were planted in a yield trial in 2006 (US application 05-305-07R).

3.3 Future prospects

In spite of the huge interest to improve yield realization and the multitude of research approaches, many of the achievements have not passed the basic research stage yet. In the case of research conducted in rice, Zhao and Zhang (2007) attributed this to a number of facts including:

- The achieved abiotic stress tolerance improvement in most transgenic rice plants was limited, insufficient to present a significant productivity increase and potential commercial success.
- Plants tolerant to abiotic stresses showed a complex trait due to impacting on a network of genes. Better insight into the complex network of genes responsible for abiotic stress tolerance is required, so that “master” genes for stress tolerance can be targeted.

Reviewing the future prospects for TFs, Century *et al.* (2008) confirmed that the key challenge is to develop leads into viable products. In particular they cite:

- Establishing whether the target pathways of that TF are present in the engineered crop, especially when a lead has been identified in a model system such as *Arabidopsis*. However, given the many examples that have been cited for *Arabidopsis* TFs functioning in heterologous systems and the evidence of conserved pathways from orthology among different species, this issue is likely to be less of a problem than might have been previously anticipated.
- Optimization of TF technologies, either to reduce unwanted side-effects such as growth retardation or to enhance the desired trait to the level at which it is of commercial value. Optimization is frequently approached by modifying expression of the TF transgene by tissue-specific, developmental, or inducible promoters, rather than the usual constitutive promoters. Another strategy for optimizing the phenotype is by protein modification.

Paterhänsel in his review on photosynthesis (2008) stressed that modifying one aspect of the system with one or two extra genes will enhance productivity to the next limiting process. It

therefore might be necessary to combine several approaches simultaneously transferring many genes to obtain the desired effect.

Flowers (2004) pointed out the misleading scientific enthusiasm in some communications and called for more experimental rigour. He illustrated this by showing that in spite of the complexity of salt tolerance, there are commonly claims in the literature that the transfer of a single or a few genes can increase the tolerance of plants to saline conditions. His evaluation of such claims revealed that, of the 68 papers produced between 1993 and early 2003, only 19 report quantitative estimates of plant growth. Of these, four papers contain quantitative data on the response of transformants and wild-type of six species without and with salinity applied in an appropriate manner. About half of all the papers report data on experiments conducted under conditions where there is little or no transpiration: such experiments may provide insights into components of tolerance, but are not grounds for claims of enhanced tolerance at the whole plant level. Whether enhanced tolerance, where properly established, is due to the chance alteration of a factor that is limiting in a complex chain or an effect on signalling remains to be elucidated.

Also Blumwald (2004) highlighted the challenge of reliable assessment of stress tolerance in plants. The assessment of stress tolerance in the laboratory often has little correlation to tolerance in the field. Also, the effect of a certain gene may differ from one species to another. In addition, field testing is difficult because of the variability of the soil (*e.g.* for salt tolerance), weather during the growing season (for drought, cold and heat tolerance) and the interactions with other environmental factors.

Looking at the biotech company's pipelines, all the 'big' crops are mentioned: maize, soybean, oilseed rape, cotton and rice.

- Monsanto is well advanced in GM applications in maize. In developing high yielding and stress tolerant varieties, Monsanto collaborates with BASF[11]. The first generation drought tolerant maize is submitted for approval in several countries and market launch in the US is anticipated around 2012. On dry land yield is announced to be 6 to 10% higher than for current varieties. The second generation is in the so-called phase II of development, as is the maize with enhanced yield. Phase II is the trait development phase where large-scale transformations are conducted and the number of candidates is reduced to some tens. Each development phase may take one to two years, but overlap is possible.
- The NUE maize project is also a combined effort of Monsanto and BASF. This trait is in the proof-of-concept phase where a number of gene leads are tested in the crop.
- For soybean Monsanto emphasizes on yield enhancement. The first generation events are in phase III undergoing field testing and regulatory data are collected. The next wave of events is in phase I for proof-of-concept. Also cotton is modified for tolerance to drought (phase I, BASF collaboration). In addition Monsanto started to invest in wheat through the acquisition of WestBred, Montana. The focus will be on drought tolerance, nitrogen use and higher yield. Analysing the field trial applications in Canada[12], Monsanto is active in oilseed rape not only to improve seed yield but also WUE and NUE. Mendel Biotechnology cooperates with Monsanto and others to commercialize its technology.
- Pioneer invests in drought tolerant and NUE maize[13]. The projected introduction for the first varieties tolerant to moderate drought is 2011. They are developed using molecular breeding techniques and are announced to yield 5 to 10% more compared to standard hybrids in dry land environments. A combination of genetic modification and molecular breeding delivers the second wave of drought tolerant maize. These will improve yield even more and are expected in 5 to 7 years. Hybrids that exhibit enhanced efficiency in nitrogen use and so require reduced quantities of nitrogen while maintaining overall yield and/or increase overall yield at existing nitrogen application levels are projected for the next decade. For soybean transgenic approaches are used to increase yield. Early-stage lead events have

[11] http://www.monsanto.com/products/pipeline/default.asp
[12] http://www.inspection.gc.ca/english/plaveg/bio/confine.shtml
[13] http://www.pioneer.com/web/site/portal/menuitem.8e36377986a91418bc0c0a03d10093a0/

demonstrated the ability to enhance soybean yield from 8 to 15% during the last three years of testing. Most of the field work for oilseed rape is carried out in Canada, trialling both yield and stress tolerance traits.

- Performance Plants Inc, a Canadian plant biotechnology company, is developing technology for commercialization, under the name Yield Protection Technology™ (YPT™), water efficiency technology™ (WET™) en heat stress technology™ (HEAT™). YPT™ is also being developed for maize, soybean, cotton, ornamentals and turf grass to be available to farmers in early 2011. In oilseed rape emphasis is on stress tolerance.
- Syngenta expects to launch its drought tolerant maize in 2011[14]. The NUE varieties will be available come before 2015, as they are still in early development.
- Bayer CropScience is currently optimizing rapeseed, rice and cotton to withstand drought and flood. The company opts for optimized photosynthesis to ensure better energy use by recycling CO_2 lost during photorespiration (Kabeish *et al*., 2007). Apart from a yield increase with oilseed rape Bayer CropScience also works on stress tolerance, more particularly on drought resistance and freezing tolerance. In Canada several field trials have been performed since 2005.
- As mentioned above, BASF collaborates with Monsanto for drought tolerance. Development work is focusing on the world's most important crops: corn, canola, soy and cotton. Crops with higher yield potential are expected to be available in the middle of the next decade. CropDesign, a subsidiary of BASF is active in improving rice for yield, drought and salt stress and NUE.
- Arcadia Biosciences is working on NUE, WUE and salt tolerance, but makes no projections for market launch.
- Targeted Growth active in bioenergy crops, focuses on yield improvement. It claims 20-40% yield increase for soybean. No estimates are given for market introduction.
- Biogemma, an affiliate of Limagrain based in France, has been working on drought tolerant maize. It is however unclear whether these events will be marketed in the near future.
- SESVanderHave, a company dedicated to sugarbeet breeding, has applied for NUE field trials for the first time in 2009 in the US. Clearly, this research will need several years to result in products.
- CSIRO delivers outstanding research in cotton and cereals such as wheat and barley, but targets mainly the Australian market.

In addition it is also of interest to note a number of projects involving public research and public-private partherships.

- In India drought and salt tolerant rice varieties are developed and are expected to be commercialized after 2015 (Stein & Rodríguez-Cerezo, 2009). The drought tolerant line has the tobacco osmotin gene inserted (Tamil Nadu Agricultural University). Tolerance to saline soils is obtained by inserting a gene for glyoxalase I and II. Dwarf potatoes (GA20 gene) are in the R&D pipeline of the Central Potato Research Institute and expected for 2012. Mannitol-1-phosphate dehydrogenase containing tomato and eggplant will presumably be commercialized from 2014 onwards. Target market for all these applications is India.
- The African Agricultural Technology Foundation (AATF) is leading a public-private partnership called Water Efficient Maize for Africa (WEMA)[15] to develop drought olerant African maize using conventional breeding, marker-assisted breeding, and biotechnology. AATF will contribute its leadership, experience in public-private partnership management, technology stewardship and project management expertise. The non-profit International Maize and Wheat Improvement Center (CIMMYT) will provide high-yielding maize varieties that are adapted to African conditions and expertise in conventional breeding and testing for drought tolerance. Monsanto will provide proprietary germplasm, advanced breeding tools and expertise, and drought tolerance transgenes developed in collaboration with BASF.
- The Improved Maize for African Soils Project (IMAS) projects to develop maize varieties that are better at capturing the small amount of fertilizer that African farmers can afford, and that use the nitrogen they take up more efficiently to produce grain. The collaboration will be led

[14] http://www.syngenta.com/en/about_syngenta/researchanddevelopment.html
[15] http://www.aatf-africa.org/wema

by CIMMYT and includes the DuPont Business, Pioneer Hi-Bred; the Kenya Agricultural Research Institute (KARI); and the South African Agricultural Research Council (ARC). IMAS participants will use conventional breeding, biotechnology tools and transgenic approaches to develop varieties that ultimately yield 30-50% more than currently available varieties, with the same amount of nitrogen fertilizer applied when grown on poor soils.

3.4 Conclusion

Many mechanisms influence the realization and the safeguarding of the production potential of a plant. In consequence each of these mechanisms are potential targets for designing approaches to improve crops. As illustrated in this section diverse ways are pursued to influence these targets. While there are many more genes and functions under investigation, this overview focussed on those applications for which at least field trials have been noted. This selection was inspired by the scope of the study being limited to those applications that may be subject to regulatory oversight in the near future (5 years).

Based on this overview some general observations can be made:

- Many of the applications mentioned in this review –although in some cases involving field trials and patent applications- may remain limited to basic research. While leading to further documentation of the molecular and physiological mechanisms underlying essential plant functions, their effect may not be adequate for being developed in a product. Table 1 provides a summary of selected traits and crops that have been deployed in field trials in the USA, Canada and Australia.

Table 1 Excerpt of Crops and Traits Currently in Field Trials in Canada, Australia, and the USA (Based on Warwick *et al.* 2009)

Trait type	Trait	Crop
Abiotic stress	Nitrogen-use efficiency	Canola, corn, rice, soybean, oilseed rape, sugarbeet, sugarcane, tomato, alfalfa, wheat, switch grass
	Drought tolerance	Corn, cotton, potato, soybean, tobacco, wheat, sweet potato, oilseed rape, tomato, poplar, sugarcane, switch grass, creeping bent grass, guayule, Bahia grass, Festuca, rice, Kentucky bluegrass, ryegrass, Bermuda grass, persimmon
	Cold tolerance	Corn, potato, soybean, Eucalyptus, Bahia grass, cotton, oilseed rape, tomato, persimmon
	Salt tolerance	Corn, rice, soybean, creeping bent grass, Bahia grass, tomato, cucumber, cotton, oilseed rape, wheat, Kentucky bluegrass, creeping bent grass, Bermuda grass, ryegrass
	Stress tolerance	Barley, canola, corn, soybean, wheat, tomato, cotton, potato
	Heat tolerance	Corn, cotton, wheat, tomato, soybean, creeping bent grass
	Flood tolerance	Rice, cotton
Plant morphology/ physiology	Fertility	Canola, corn, Eucalyptus, switch grass
	Altered maturity/development	Canola, corn, soybean, rice, tomato, loblolly pine, Kentucky bluegrass, creeping bent grass, poplar, sweetgum
	Altered senescence	Corn
	Yield increase	Alfalfa, canola, corn, rice, soybean, tobacco, wheat, alfalfa, peanut, cotton, guayule, camelina, tomato, lettuce, falseflax
	Altered flowering time	Corn, plum, poplar, tomato, tobacco, melon, soybean, alfalfa, strawberry, apple, walnut
	Germination increase	Canola, corn

- Whereas first biotechnology products were based on 'simple' monogenic traits, such as herbicide tolerance or insect resistance, these developments are typically building on plant-own mechanisms and/or require influencing complex biochemical pathways. Often genes are taken from plant species, sometimes from the same species. Similarity with other naturally occurring changes (*e.g.* mutation) has been pointed out by several authors.

- Stress responses in plants show high levels of complexity and redundancy at the perception, signalling and expression levels with cross regulation ("cross-talk") between stress pathways and overlapping functions between stress metabolites and stress proteins in different stresses. Each of these is a potential target for improving the yield realization under specific conditions. Molecular approaches vary from expressing new functions, to over-expression and silencing of plant-own functions.

- Especially when the modified factors intervene in different plant processes (*e.g.* in the case of plant hormones or signalling functions), complex and sometimes undesired effects are observed. In consequence, the inserted function is very often controlled by a promoter that limits the expression to specific plant parts, development stages and or growing conditions (*e.g.* when the stress factor is present).

- Some applications are targeted to increasing the yield potential whereas others aim to provide yield stability under sub-optimal conditions (the latter are typically referred to as abiotic stress tolerance). There are very few reports on yield securing strategies that also enable yield improvement under optimal conditions.

- In the study of stress, researchers historically have tended to specialize in the study of specific stresses which has resulted in a narrow perspective on this phenomenon. Current elucidation of stress responses suggest that there is cross induction ("cross-talk") in the stress signalling pathways between the specific stress responses. Plants may respond to stress perception by an initial global response ("stress cross signalling") involving initially activation of a global stress response with elements of an oxidative, a "heat shock" and a "pathogenesis" stress response and followed by a more specific or customized stress response specific to the cues abiotic or biotic perceived.

- Another complicating factor is that under realistic field conditions, a plant is constantly subjected to many stress factors that act in different degrees. As a consequence, the plants' own defence systems will be active at some level. Any engineered strategy will act in addition to and/or in interaction with the already available mechanisms.

- The large diversity of targets and strategies makes referring to the trait as abiotic stress tolerance misleading. It might create a perception that plants are produced that are able to successfully withstand a combination of stress factors. On the contrary, typically only a very specific stress response is targeted. Even taking into account the phenomenon of cross-talk, the effect of a single strategy is usually limited to specific effects in the stress reaction of the plant.

- While the architecture or stress response may have changed (*e.g.* dwarf growth, increased number of tillers), the overall aspect of the modified plants as well as their developmental and biological characteristics is usually maintained. There are for instance no reports that a self-pollinating crop changed to obligate cross-pollination as a side-effect of a modification targeted for a stress tolerance.

- Although a substantial number of biotechnology product opportunities clearly exist, particularly when all of the different types of crops are considered, many of these represent relative niche markets, which would not justify the very high costs that are currently associated with GM product development. For this reason, significant commercial efforts are primarily focused on increasing intrinsic yield potential and stabilizing yield in the face of environmental pressures in large-acre row crops.

4 Environmental Risk Assessment – Legislation, guidance & approvals

In line with the precautionary principle, the European legal framework for activities with GMOs requires that an Environmental Risk Assessment (ERA) is conducted prior to the onset of such activity. The scope of an ERA is broad and covers potential impacts on the environment, plants, animals and human health. In this report aspects related to food and feed use have been omitted.

4.1 European & national legislation

Directive 2001/18/EC[16] on the deliberate release into the environment of genetically modified organisms imposes that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release or the placing on the market of GMOs. In the Netherlands, this Directive is implemented by the GMO Decree[17]:
The ERA is further elaborated in Commission Decision No 2002/623/EC[18]. Regulation (EC) No 1829/2003[19] on genetically modified food and feed refers to Directive 2001/18/EC concerning the ERA (definition and conducting).

The objective of an ERA is, on a case-by-case basis, to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may cause. The ERA should be conducted with a view to identify if there is a need for risk management and if so, the most appropriate methods to be used.

As part of the ERA, the characteristics of the GMO have to be compared to those presented by the non-modified organism in a scientifically sound and transparent manner and taking into account:
- the recipient or parental organism(s);
- the genetic modification(s), vector and the donor;
- the GMO;
- the intended release or use including its scale;
- the potential receiving environment; and
- the interaction between those.

The analysis in this report will be performed against the background of European legislation, as COGEM will evaluate dossiers submitted in the Netherlands and the European Union. However, opinions on dossiers by authorities outside Europe can be helpful in assessing the specificities for these types of GM plants.

4.2 Technical guidance

In addition to the Legal instruments, there are also technical guidance documents, reports and discussions in regulatory platforms that are relevant for scoping the ERA for yield enhancing and yield securing traits with Annex II of Directive 2001/18/EC on the principles for the ERA as a starting point.

---

[16] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L106/1, 17.4.2001)
[17] GMO Decree: Decree of January 25, 1990, laying down a general measure of governance under Article 24 of the Chemical Substances Act (last amended September 3, 2004)
[18] Commission Decision No 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L200/22, 30.7.2002)
[19] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L268/1, 18.10.2003)

4.2.1 EFSA guidance documents

EFSA's core task is to independently assess any possible risks of GMOs to human and animal health and the environment. EFSA does not authorize GMOs, which is done by the European Commission and Member States in their role as risk managers. EFSA's role is strictly limited to giving scientific advice.

EFSA's GMO Panel has prepared guidance documents in line with the regulatory framework. These concern the type of data that applicants must include in GMO applications and the risk assessment approach applied by the GMO Panel. The main general guidance document concerns EFSA's overall risk assessment approach for GM plants and derived food and feed (EFSA, 2006).

4.2.2 Other guidance documents

4.2.2.1 US National Research Council

In 2002 the US National Research Council issued a report on the environmental effects of transgenic plants, which was drafted by its Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants (NRC, 2002). One section of this report focused specifically on the assessment of potential environmental risks posed by GM plants with tolerance to abiotic stress. The Committee anticipated that an accurate assessment of the environmental risks posed by any of these stress-tolerant plants will not be possible until they are actually created because the genetic mechanism of stress tolerance will greatly determine the scope of potential risks.

Despite this uncertainty regarding the nature of stress-tolerant transgenic plants, the Committee commented that these developments have raised concerns about environmental risks. Abiotic conditions, such as soil nutrient levels, water, cold, heat, salt, and metal toxicity, combined with their seasonal variations have strong determining effects on plant community structure worldwide, and the geographic distribution of many plant species is influenced strongly by these factors. Thus, when plants are transformed to better tolerate these abiotic conditions, it raises risk questions about the possibility of impacts on plant community structure and expansion of the geographic range of a plant species.

The Committee pointed out that environmental risks associated with such stress-tolerant crops are both complicated and subtle. To clarify their analysis, the Committee focused on drought tolerant crops, in particular examples based on higher water use efficiency (WUE), which leads to greater biomass production per unit of water, or an increased ability to extract water from the soil. These examples show that the environmental effects of these mechanisms will very likely differ, namely:

- Plants with improved water extraction are expected to require the same amount of water to grow, so potential environmental effects may often be related to competition for sunlight or nutrients in the soil due to the plant's metabolic needs associated with greater biomass.
- In contrast, ecological theory suggests that a plant with a higher WUE would be predicted to be a better competitor for water than a non-transformed plant. This hypothesized improved competitive ability is the source of some concerns about the environmental risks of drought tolerant transgenic plants, whether it is the crop or a wild relative that might receive the transgenes by horizontal gene flow.

According to the Committee, increased competitive ability for water is not necessarily sufficient to cause a plant to expand its geographic range. The Committee further stressed that assessment of these risks will require attention to the plant, trait, and environment. In a similar way, impacts on plant community structure and non-target species and interactions among traits might occur, but their assessment will be case specific. For example, certain transgenic salt-tolerant plants can also tolerate other stresses including chilling, freezing, heat, and drought.

The report also discussed the environmental hazard of a GM plant as a whole due to a transgenic trait that may improve its fitness and ecological performance. In this context it is noted

that many crop plants may pose little risk, in so far as they are unable to survive without human assistance, also because crop plants frequently have characteristics, like lack of seed shattering and seed dormancy, which make them useful to humans but also reduce their ability to establish feral populations in either agro-ecosystems or non-agricultural habitats. Without major changes in its phenotype, maize is unlikely to survive for multiple generations outside agricultural fields, no matter what transgene is added to it.

4.2.2.2 Ad Hoc Technical Expert Group on Risk Assessment and Risk Management

At its fourth meeting, the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP), in its decision BS-IV/11[20] , established an Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management (RARM) under the Cartagena Protocol on Biosafety. The results of the work of the AHTEG, will be reported to the fifth meeting of the Parties, to be held in October 2010. The results have already been made publicly available through the Biosafety Clearing House.

As outlined in the annex to decision BS-IV/11 the mandate of the AHTEG on RARM includes:

- Developing a "roadmap", such as a flowchart, on the necessary steps to conduct a risk assessment in accordance with Annex III to the Protocol and, for each of these steps, provide examples of relevant guidance documents;
- Taking into consideration the identified need for further guidance on specific aspects of risk assessment, including particular types of (i) living modified organisms (for example, fish, invertebrates, trees, pharmaplants and algae); (ii) introduced traits; and (iii) receiving environments, as well as monitoring of the long-term effects of living modified organisms released in the environment, prioritize the need for further guidance on specific aspects of risk assessment and define which such aspects should be addressed first, taking also into account the need for and relevance of such guidance, and availability of scientific information.

During the first meeting of the AHTEG in Montreal from 20 to 24 April 2009, the Group agreed to establish sub-groups to focus on specific issues, including one Sub-Working Group (SWG) on the development of a roadmap for risk assessment; and one SWG on living modified crops resistant or tolerant to abiotic stress. Each SWG received input from a Discussion Group comprised of experts. One of the deliverables of the SWG is a guidance document on its topic.

During the preparatory discussion of experts and in Annex, II Part B. “Risk Assessment Of Living Modified Crops With Tolerance To Abiotic Stress” of the final report of the AHTEG[21], the following points were highlighted.

- The definition of abiotic stresses is usually overstated and should be recalibrated based on experience with traditionally bred crops. It should be acknowledged that plants are under stress constantly.
- Imprecise terms like "drought", "heat" and "cold" are descriptive, but may not be descriptive or precise enough to conduct a risk assessment and should therefore be handled cautiously.
- The importance of carefully constructing hypotheses that account for the intended differences is highlighted. For instance it is questioned how to test for a drought tolerant crop the hypothesis that the GM crop would be phenotypically unchanged compared to the non-GM crop when water is limited and when water is optimal.
- The risks associated with abiotic stress tolerance can be assessed in the same way as that of other types of genetically engineered crop plants, following the steps for risk assessment in Annex III in the protocol.
- The first step is to identify adverse effects that may be associated with any novel genotypic and phenotypic changes associated with the abiotic stress tolerant Living Modified Organism (“LMO”). By comparing the LMO to its traditional counterpart, any novel changes associated with the abiotic stress tolerance can be identified, including any changes to the biology of the

[20] http://bch.cbd.int/protocol/decisions/decision.shtml?decisionID=11690%20
[21] http://www.cbd.int/doc/meetings/bs/bsrarm-02/official/bsrarm-02-05-en.pdf

crop plant (*e.g.* if the genes may alter multiple characteristics of the plant) or to the potential receiving environment (*e.g.* if the plant can grow where it has not been grown before).
- One of the main challenges seems to be the selection of the right "comparator(s)". Although the abiotic stress tolerance of the LMO may result only in a relative improvement of the stress response, the LMO will be able to grow under conditions where the growth of the typical comparator may be impaired. This will complicate a real comparison under relevant conditions. Ensuring that the entries are tested under diverse conditions, including varying degrees of the stress factor(s), will be important to draw conclusions on similarity.
- The potential for cross-talk between abiotic and biotic stress mechanisms in the LMO can be identified in the characterization of the novel gene products.
- After adverse effects associated with the introduced changes have been identified, then the likelihood and consequences can be considered together to determine the risk and the need for any additional risk management.
- "Fitness" is the ultimate measure of a plant's performance. It is one of the assessment items of "adaptability to abiotic stressed condition" in this context. Concepts like "fitness" need to be kept in perspective of risk assessment. There is much coming out in the literature about how stress traits enhance fitness. However, the question for the risk assessment is not whether a plant is more fit, but whether it becomes a weed/pest, unacceptably invasive, etc. as a result of the genetic modification. A change in fitness should not be construed as representing harm *per se*.

4.3 Precedents

4.3.1 Commercial releases

4.3.1.1 Drought resistant maize

Monsanto has applied for commercial release of transformation event MON 87460 in USA (FDA, APHIS 09-055-01p), in Canada[22], in the Philippines[23] Australia/New Zealand (FSANZ: Application A1029) and in the EU (EFSA-GMO-NL-2009-70). The EU application, submitted via the Netherlands according to Regulation (EC) No 1829/2003 on genetically modified food and feed, covers all uses except seeds and propagating material for cultivation in the EU.

At the time of completing this report, few details on the product and the risk assessment were available.The following information was taken from the Summary Notification Information Format (SNIF) as submitted by Monsanto[24]:
- MON 87460 (OECD Unique Identifier: MON-8746Ø-4) expresses cold shock protein B (CspB) from *Bacillus subtilis* and NptII from Tn5 of *Escherichia coli*.
- MON 87460 was developed to provide reduced yield loss under water-limited conditions compared to conventional maize.
- CspB is an extensively studied protein known to facilitate adaptation to environmental stresses in bacteria. CspB is known to bind and unfold secondary RNA structures that compromise the ability of the cell to translate those RNA molecules, thus helping to preserve normal cellular functions. Expression of CspB is controlled by the promoter and leader from the rice actin gene (P-*Ract1*).
- Under well-watered conditions, grain yield for MON 87460 is equivalent to conventional maize. Under water-limited conditions, grain yield loss is reduced compared to conventional maize. However, like conventional maize, MON 87460 is still subject to yield loss under water-limited conditions, particularly during flowering and grain fill periods when maize yield potential is most sensitive to stress, by disrupting kernel development. Under severe water deficit, maize grain yield for MON 87460, as well as conventional maize, can be reduced to zero.

[22] http://www.inspection.gc.ca/english/plaveg/bio/subs/2009/20090324e.shtml
[23] http://biotech.da.gov.ph/Decision_docs_direct.php
[24] http://www.gmo-compass.org/pdf/regulation/maize/MON87460_maize_application.pdf

- Comparative assessments in the field are claimed not to reveal any biologically significant differences between MON 87460 and conventional maize hybrids, except for the introduced trait that is of agronomic interest.
- Compared with conventional maize, the presence of the drought tolerance trait is only expected to confer a selective advantage where water-limiting conditions are present at levels that would suppress yield, and if no other, more important factors limiting the survival of maize in the receiving environment would be present. In practice, however, this advantage is deemed to be of limited consequence because of the poor survival characteristics of maize under most European conditions and since the trait was shown not to provide a meaningful selective advantage or disadvantage that altered the survival of MON 87460 maize as volunteer plants or in areas that outside of agricultural production.
- In the SNIF there is no indication on cross-talk, although it is known that the function of the protein in its host (*B. subtilus*) is to facilitate adaptation to environmental stresses. Nickson (2008) commented that tolerance to drought might have a small, detectable change in response to another stress, *e.g.* heat due to low levels of cross-talk with other stress response pathways. He argues that such other stress response is likely not be commercially useful because it is low magnitude and inconsistent across regions.

4.3.1.2 Freeze tolerant eucalypt

ArborGen has submitted a petition for freeze tolerant *Eucalyptus* transformation event ARB-FTE1-08 to the USDA (APHIS 08-366-01p). The event has also been marked as carrying an altered fertility. ArborGen claims that its freeze tolerant eucalypts can tolerate most severe drops in temperature and also excels at biomass production. At the moment of this survey, no details on this application were available.

However, APHIS assessed the risk of cold tolerant *Eucalyptus* field trials (APHIS, 2007, 2009a and 2009b). Given the parent crop characteristics (insect pollination, small seeds that germinate only under special conditions, no wind dispersal of seeds, no dormancy, sensitive to weed competition, intolerant of shade, limited sexual compatibility, self incompatibility; and not native to the US) APHIS considered it very unlikely that the introduction of these genes would make *Eucalyptus* invasive or weedy. The *Eucalyptus* species that is used has difficulty establishing without human intervention, even in warm climates. No effect on pests, pathogens or other non-target organisms is seen in previous non-flowering trials. The *CBF* gene is under the control of a cold-inducible promoter that allows for normal growth under non-stress conditions.

4.3.2 Field trials

In field trials often early development events are tested for proof-of-concept, for which not many data are available regarding phenotype. Often information from the literature on related genes and species is used to identify potential risks to be considered. In this overview some elements are discussed that relate primarily to the introduced genes and their potential effects. While the ERA information may provide insights in the approach taken by the different authorities, it has to be considered that trial confinement measures may largely influence the risk assessment. In such case, the ERA may not address the essence of a potential impact, but rather focus on how the confinement measure reduces the likelihood for certain potential interactions to occur.

4.3.2.1 USA

Apart from the ERAs for cold tolerant *Eucalyptus* trials described above, no reports are published assessing the risks for stress or yield-enhanced crops.

4.3.2.2 Australia

OGTR analysed the applications for yield (plant architecture), drought tolerant and NUE enhanced **sugarcane** and concluded that the limited and controlled release of these GM lines poses a negligible risk to the environment (OGTR, 2007a and 2009b).

They considered that potential changes resulting from modifying plant growth could include:

- more vigourous growth improving the ability of sugarcane to establish in a competitive environment;
- increased plant height improving the ability of sugarcane to shade out other plants;
- shorter stature resulting in a decreased tendency to lodge;
- altered bud growth characteristics leading to stem pieces more readily shooting and establishing as new plants; and
- potentially affected fertility, flowering time and seed development.

Also, improved NUE could increase weediness in an environment in which nitrogen availability is the main limiting factor. The *ZmDof1*gene responsible for NUE is also involved in numerous other processes including light responses, auxin responses, defence and seed germination (Yanagisawa *et al*., 2002). *OsDREB1A* could not only effect drought tolerance but also salt and freezing tolerance due to interconnected signalling and transcriptional controls.

OGTR stated that it is unlikely that introduction of these genes could alter all of the characteristics which limit the spread and persistence of sugarcane such as its low fertility and seed viability. Although alteration of plant architecture could potentially result in weediness of the GM sugarcane lines, it could also result in GM plants of lower fitness compared to other commercially available sugarcane varieties. Increased plant height may in turn result in an increased susceptibility to lodging and decreased height may make the plant prone to shading by competition.
OGTR expected that unintended pleiotropic effects had most likely been detected already in the pre-trial phase. The uncertainties that could still have existed were deemed inherent to early development trials and are anticipated by containment measures and monitoring.

Application DIR053 covered testing the effect of the ornithine aminotransferase gene in **wheat** for salt tolerance. In greenhouse conditions the GM plants, compared to wild-type plants, accumulated 3 times as much proline serving as an osmoprotectant (OGTR, 2005). The plants were slightly smaller probably because of the metabolic drag due to the constitutive expression, reducing the fitness. Proline and the enzyme are ubiquitous and are not toxic or allergenic to wildlife that would feed on the plants even at higher concentrations. Elevated proline levels are also thought to confer tolerance to some other environmental stresses, including frost and moisture stress. However, although the gene is constitutively expressed, the proline level is still subject to feedback regulation and catabolism. Also, there is often little correlation between laboratory and field results (Blumwald *et al*., 2004) as is argued in the OGTR risk assessment and risk management plan. It was concluded that, while the genetic modification may provide the GM wheat with an advantage in some environmental conditions relative to non-GM wheat, it was unlikely to increase other characteristics normally associated with intrinsic weediness.

The ERAs of DIR071 and DIR080 mentioned that the genes for drought tolerance introduced in wheat were isolated from plants, a moss and yeast (OGTR, 2007b and 2008b). They have been shown in *A. thaliana*, canola and/or maize to confer increased WUE or tolerance to water stress. The signalling pathways for abiotic stress tolerance are not strictly isolated. The regulatory nature of some of the introduced genes for drought tolerance may mean that the encoded proteins could also confer tolerance to other environmental stresses, such as extremes of temperature or soil salinity. Furthermore, it was anticipated that the GM wheat lines could possess other characteristics under stress such as increased seed dormancy, viability, or improved seedling germination rates which may impact their weediness potential. They could also impact biotic stress tolerance. Even when taking these into consideration, OGTR did not consider these traits a risk for wheat to become a weed.

The **GM wheat and GM barley** lines in DIR077 contained one of two drought responsive transcription factors (TaDREB2 and TaDREB3) derived from wheat (OGTR, 2008a). In the greenhouse a semi-dwarf phenotype was observed in some wheat and barley plants modified with the *TaDREB2* or *TaDREB3.* In addition, the DREB2 family of proteins provided tolerance to both saline soils and drought.
Another abiotic stress tolerance transcription factor derived from wheat has been introduced into other barley lines. These lines had larger spikes and seeds and delayed growth and development compared to the wild type control plants when grown in greenhouse conditions.

The identity of the gene for a higher NUE in DIR094 was not disclosed (OGTR, 2009a), except for its origin species which is barley. OGTR considered the GM wheat and barley not able to establish and persist in the environment. Both cereals are wind pollinators but lack other characteristics of weeds making reference to Keeler (1989). The modification would contribute only incrementally to the potential weediness, spread and persistence.
Among the possible effects that were considered a few are related to the actual modification:

- as a result of the enhanced NUE the GM lines could deplete nitrogen from soil and 'starve' surrounding vegetation;
- the GM wheat and barley lines may show an increase in total nitrogen content;
- nitrogen content in wheat seeds can affect dormancy;
- high level of nitrogen fertilization has been shown to increase pre-harvest sprouting.
- possibly this could also lead to reduced dormancy in the GM lines, but this is clearly a disadvantage for the crop.

The GM **torenia** lines that have been modified to enhance their capacity to absorb phosphate raised similar questions (OGTR, 2008e). The gene could be linked with improved survival and could therefore have a selective advantage in soils where phosphorus is a limiting factor for plant growth. Activation of the phosphate starvation response in plants generally increases secondary metabolism leading to enhanced flavonoid (*e.g.* anthocyanins) and indole alkaloid production thereby enhancing pest and disease resistance or tolerance to stress.

Genes originating from several plant species were transferred separately to **cotton** to improve water stress tolerance (OGTR, 2006a and 2008c). The genes encode proteins that modulate biochemical pathways, function as molecular chaperones, or regulate signal transduction and expression of endogenous genes in the cotton plants. The exact nature of the genes is kept confidential. Again, the drought tolerance trait was judged as not sufficient to enhance the spread and persistence of cotton.

Homologues of the introduced genes are known to enhance another abiotic stress tolerance other than drought stress tolerance such as cold, freezing, pathogen, salinity, nutrient deficiency, and heavy metals.

- One gene has the ability to also enhance tolerance to fungal cotton disease. But, the exact mechanism is not known. OGTR made a comparison with conventionally bred varieties that show tolerance to the same fungus without giving weedy problems.
- Other genes could potentially affect fertility and alter flowering time, or are involved in flower morphology and pollen development. In a field trial a difference in flowering time compared to the surrounding non-GM border would compromise its function as a pollen trap. This could be anticipated in the permit requirements. In the first field trial, although preliminary, no significant differences in flowering time were observed.
- Homologues of some of the other introduced genes have been shown to be involved in seed germination, embryo development, nutrition in developing seeds, seed development and desiccation tolerance in the developing seed. These anticipated effects are drawn from the literature and probably not all, if any, were expected to eventuate. Also, they do not necessarily find expression in other plant species (see *e.g.*: Oh *et al*., 2005).

OGTR concluded that there was no indication from the literature that the introduced genes could alter all of the characteristics which limit the spread and persistence of cotton such as seed dormancy, seed persistence in soil, length of life cycle, large amount of seed dispersed and long-distance dispersal of seeds. In addition, it was argued that a GM plant that is resistant to multiple stresses may be less fit because of associated metabolic/physiological burdens.

OGTR also permitted a release of GM cotton plants with tolerance to waterlogging (OGTR, 2006b and 2008d). The inserted gene encodes one of the non-symbiotic haemoglobins (nsHbs) that are widely distributed in the plant kingdom. The introduction of an nsHb gene has been shown to affect a number of different pathways in plants such as response to pathogen infection or symbiotic interactions, enhanced seedling growth rates and root architecture. Over-expression of the *AHb1* gene in GM *Arabidopsis* leads to better resistance to waterlogging and also to

enhanced early growth rates under normal growth conditions due to increased size of roots and shoots. This is not observed in tobacco. Natural nsHbs are induced in cotton during fungal infection in *Fusarium* or *Verticillium* susceptible plants. These reactions are not consistently found in other plant-fungus combinations. In laboratory experiments in *Arabidopsis*, *AHb1* gene expression responds to nitrogen fertilizer, nickel exposure, high CO_2, ethylene and heat stress.

In DIR083 the *AHb1* gene is combined with *Pdc2* and *Adh*. PDC and ADH provide an alternative metabolic pathway for energy production under low oxygen conditions (hypoxia and anoxia). Over-expression of these genes leads to higher ethanol and acetaldehyde levels. Both compounds are widespread in plants, especially in fruits, and in the environment. They may be toxic above certain levels. Increased acetaldehyde levels will impair the viability of plants, as the stress phenotype in waterlogged plants is thought to result from acetaldehyde accumulation. Ethanol leaching into the rhizosphere may have an effect on fauna of the immediate soil environment. However, this would also be true for non-GM cotton exposed to flooding.

OGTR considered that waterlogging is not one of the main limiting factors for cotton in the natural environment. In Australia, waterlogging of cotton is a problem due to the furrow irrigation system. Therefore, the GM trait itself was deemed not to add to the survival and persistence of cotton outside normal cropping regions.

4.3.2.3 France

Schenkelaars (2007) already mentioned two ERAs for drought tolerant **maize** developed by Biogemma. The positive evaluation is based on the fact that both applications are about plant genes already abundantly present and that maize has no relatives in Europe, is frost sensitive and volunteers, if any, are easily controlled.

The same rationale was used for all other applications by Biogemma (CGB, 2003, 2005a, 2005b, 2006a, 2006b, 2006c and 2006d). All genes are derived from plants or even maize itself. None of the expressed proteins is considered a risk for human health or environment. It was concluded that there was no risk for maize becoming a weed or for outcrossing.

4.3.2.4 Spain

In the ERAs for field trials with transgenic **citrange and orange** trees emphasis was put on the nature of the recipient plants that are multiplied vegetatively (grafting) and on reducing the likelihood for outcrossing by a border row and by avoiding honeybees, as is practised in commercial plantations (MMA, 2007, 2008a and 2008b). Therefore and based on observations in a previous trial with marker genes the GM trees in the field trial were deemed not to present any risk for establishment and dissemination. However, the Comisión Nacional de Bioseguridad (CNB) recommended to study the potential effects on other organisms during the trial, more particularly the effect on trophic chains (CNB, 2008).

For heat tolerant **potatoes** a similar rationale was used (MMA, 2009). The plants were planned to grow and flower in a period when no commercial crop was grown due to the high temperatures. The CNB assessed that this would not affect survival, establishment, dissemination or the way of reproduction. Again the CNB recommended taking the opportunity to study potential effects on biodiversity near the release site.

4.3.2.5 Germany

The final report of the **potato** trials for WUE (B/DE/04/159) mentions a slight growth retardation early in plant development for the GM plants compared to the parent plants[25]. The final tuber yield was the same or lower than for the wild type plants. No differences were noticed in flowering characteristics or pest and disease resistance.

[25] http://gmoinfo.jrc.ec.europa.eu/finalreports/B-DE-04-159-Final-Report-DE.pdf

Field trials under notification B/DE/05/175 with potatoes for increased yield did not show any unexpected events, or changes in the interaction with other organisms[26]. The tuber yield was slightly lower for the GM plants and the starch level remained the same compared to the parent plants. The number of berries was lower for the GM plants indicating stronger sink strength of the tubers. It was hypothesized that due to the early diversion of photo-assimilates to the tubers, not enough green mass was developed to support further growth.

[26] http://gmoinfo.jrc.ec.europa.eu/finalreports/B-DE-05-175_final_report.pdf

5 Environmental Risk Assessment & problem formulation

Several authors point out that developing (abiotic) stress tolerance in plants will require special approaches for the ERA. *E.g.* in a review by Cassells & Doyle (2003) on genetic engineering and mutation breeding for tolerance to abiotic and biotic stresses, environmental as well as food/feed safety are addressed. However, the authors only refer to precedents of and differences with herbicide and insect tolerance traits and fail to identify issue specific for stress tolerant plants.

Wilkinson & Tepfer (2009) confirm such a specific focus and identify two justifications for a special approach:

- Some strategies for creating resistance/tolerance to abiotic stresses are based on significant changes in the plant's metabolism, and thus may require even more complex data sets for risk assessment. In contrast, early examples of herbicide and insect tolerance were mediated by proteins that are not expected to have significant direct or indirect effects on the physiology and metabolism of the plant.
- Given that traits conferring abiotic stress tolerance, such as drought or salt tolerance and the capability to withstand biotic stresses, such as disease resistance or herbivory, have been associated with selection in natural habitats, it seems entirely plausible that these plants could exhibit advantageous characteristics across a range of settings outside the farmed environment, giving rise to "ecological release"[27].

Other authors, *e.g.* Strauss (2003), acknowledge that crops engineered to improve abiotic stress tolerance based on genes from plant sources, would appear to pose a higher risk of spread in the environment than domestication traits, but point out that physiological considerations and breeding experience suggest that this might not be the case. Indeed, modifications that alter the function or expression of key regulatory genes are under strong stabilizing selection due to natural selection. They often have complex antagonistic effects on other dimensions of fitness. On this basis, they even project that the evaluation of field trial applications should be more straightforward than for the first-generation biotechnology crops.

Within the objectives of this study, this section addresses aspects of the ERA that might be specific or different for yield enhancing and yield securing traits. As for any other GMO a data package and ERA will be required according to the applicable legislation and guidance documents. Topics that need to be addressed but that are not related to the yield related traits are not covered in the scope of this report. Therefore the considerations reported here should not be regarded as the only ones to address when performing the ERA for a specific stress tolerant plant. Equally, given the large diversity of traits and strategies to achieve certain stress tolerances; this overview can only be of a general nature. Depending on the specific case, some considerations may not be relevant.

In this project the focus was specifically on issues that are relevant for the ERA. The research community may whish to develop further insights in the molecular mode of action of stress response in plants. While the value of more background information is not debated, the scope of this project relates to issues and approaches that are relevant for conducting an ERA. Similarly, while many aspects of yield enhancement and stress responses still need to be elucidated, some processes are understood and can be applied in product development. In the ERA the risk assessor will focus on the specific genetic approach, rather than considering the diversity of approaches that could lead *e.g.* to a stress tolerance.

A variety of national, regional, and international approaches to ERA of GM plants are emerging (Hill, 2005), and these contain differing legislative triggers, terminology, and guidance regarding

[27] Ecological release occurs when a species expands its habitat and resource utilization into areas of lower species diversity (Losos & De Queiroz, 1997).

how the assessments are to be performed. As this study was conducted in the scope of the European and in particular the Dutch legal framework, reference will be made to the items listed in Annex II.D.2 of Council Directive 2001/18/EC that have to be addressed in the conclusion on the potential environmental impact from the release or the placing on the market of genetically modified higher plants. In section 5.2 these items are reviewed based on issues related to the traits considered in this report.

Several papers provide a critical outlook and a structured, rational approach for the ERA of GMOs (*e.g.* Gray, 2004; Raybould, 2006; Nickson, 2008; Wolt *et al.*, 2009). According to this approach, an ERA has to start with problem formulation. At the core of the problem formulation process is the establishment of the ERA's parameters (problem context) and the identification of risks of greatest relevance (problem definition). Without further developing this approach in detail, it is relevant to recognize that among the factors that should be considered in problem definition, the nature, magnitude, and significance of the changes in the GM plant are of primary importance since they will direct the course of actions required for risk characterization.

Detected meaningful differences are then subjected to a more detailed risk assessment. The risk will be evaluated comparatively; *i.e.* on the basis of a hypothesis of no biologically meaningful differences associated with the GM phenotype versus the non transformed comparator that could produce an adverse effect or on the basis of no environmentally relevant difference in the system where the GM phenotype is released. The items included in the Annex II of Council Directive 2001/18/EC provide an indication of risk areas that need to be considered. They do not present actual protection goals or endpoints within the concept of a systematic ERA. While in this report the information is presented according to these topics, it is not the intention to substitute for an actual ERA.

5.1 Plant characterization

Nickson (2008) describes that an extensive characterization of the product that includes appropriate expression and molecular analyses as well as a detailed assessment of the plant in the field and compositional components, is fundamental to any analysis of a GM crop. Plant characterization is another term for this detailed assessment of a crop in the field. The purpose of plant characterization is to confirm or falsify the hypothesis that the GM crop is not different compared to the non-GM crop other than the presence of the introduced gene(s), the expression of the gene(s), and the intended phenotype. As such, plant characterization is designed to define meaningful differences between the GM crop and its conventional counterpart.

Typically, plant characterization uses parameters that are familiar to plant breeders and experts familiar with the crop so as to leverage expert judgment in regard to understanding differences between the GM crop and its conventional counterpart (comparative assessment). In this way data analysis can leverage both quantitative information as well as a wealth of experience from traditional breeding and from the use of the crop in agriculture. Similarly a comparative compositional analysis of key food and feed nutrients and anti-nutrients is an essential requirement for all GM crops. In addition to the relevance for food and feed safety, meaningful changes in plant composition could indicate altered ecological interactions of the plant with the biotic community, particularly interactions with pests. The implications of meaningful differences identified in the plant characterization subsequently are considered in more detail in the risk assessment.

Irrespective of this general approach, the following considerations are relevant when preparing the characterization of GM abiotic stress tolerant plants.

5.1.1 New mode of action requires new characterization approaches

To date, the vast majority of phenotypes that have been assessed have been traits for herbicide tolerance and insect resistance. They follow the simple paradigm that an introduced genetic sequence encodes a protein that results in an additional function in the GM plant. In consequence, the safety assessments have focussed on the nature and origin of the gene, the

specifics of the protein and the effect of the new function. This can include the safety of new metabolites that are produced as a consequence of the new function.

This approach could probably be maintained in case of over-expression of an existing function, although it may be considerably more challenging to monitor functions that are inherent to the plant than to observe functions obtained from other origins.

Several new approaches will drastically digress from this initial paradigm. This divergence is not specific for abiotic stress tolerance traits and it can be expected that other traits (e.g. crop quality traits) may be based on similar innovative strategies. The following are a few example that have been described in more detail in previous chapters:

- RNAi is a form of post-transcriptional gene silencing, controlling the activity of other genetic functions (*e.g.* Waterhouse & Helliwall, 2003; Baulcombe, 2004). The effect is mediated by small RNA molecules, not requiring the production of a peptide. RNAi constructs do not interfere in gene expression in other species. In plants, RNAi constructs can give rise to off-target silencing effects, where small RNAs derived from the sequence directing RNAi closely match non-target sequences expressed in the same cells. Homology of as little as 20 nucleotides can give rise to off-target silencing (Small, 2007).
- A TF is a protein that binds to specific DNA sequences and thereby controls the transcription of genetic information from DNA to mRNA. TFs perform this function alone or with other proteins in a complex, by promoting (as an activator), or blocking (as a repressor) the recruitment of RNA polymerase to specific genes. As master switches for major regulatory networks and their prior role in the domestication of many crop species, TFs are predicted to be among the best and safest candidate loci for engineering these traits (Century *et al*., 2008).
- Chaperones are proteins that assist the non-covalent folding or unfolding and the assembly or disassembly of other macromolecular structures, but do not occur in these structures when the latter are performing their normal biological functions.

Nickson (2008) questions whether detailed knowledge of the mechanism of action is needed to assess the safety of the product. For insect protected products based on proteins from *Bacillus thuringiensis*, knowledge of the mechanism is relevant to building the ERA conceptual model. In this case, Bt proteins are specifically toxic to certain pests and pose minimal risk to other organisms based on their mode of action and levels of expression in plants. On the contrary, if a gene conferring drought tolerance has no reasonable mechanism for conferring toxicity to organisms, then this information may be sufficient to conduct the ERA. Detailed knowledge of the mechanism by which a gene confers drought tolerance is unlikely to provide additional relevant insights on toxicity and may therefore not be necessary for the ERA. Knowledge that a plant is tolerant to water stress may be sufficient to guide the development of the conceptual model for the ERA.

5.1.2 Complex phenotypes

While the introduced sequences may be limited, the resulting phenotypic changes may be complex. This is due to the fact that some traits influence different reaction pathways or can effect different responses. Any modification that could have an effect on the environmental impact needs to be identified at the start of the ERA. Yet, given the complexity of some effects, it will be challenging in some cases to get a complete description. In other cases, especially when there are only few main effects and some additional minor effects, it may be sufficient to focus on those that are of significance.

5.1.2.1 Complex traits and side-effects

Plants exhibit a variety of responses to abiotic stresses that enable them to tolerate and survive adverse conditions. As we learn more about the signalling pathways leading to these responses, it is becoming clear that they constitute a network that is interconnected at many levels.

Sometimes the term “pleiotropy” is used to refer to the effect of one particular gene on other genes to produce apparently unrelated, multiple phenotypic traits (Kahl, 2001; FAO, 2001). It is a

general point of attention when developing plant products including GM products and is therefore also relevant for GMOs harbouring a stress tolerance.

Influencing plant regulatory mechanisms often results in a diversity of phenotypic effects. There may be a causal relationship between the different effects as demonstrated by the example of selection for dwarfism genes in classical breeding, a key enabling technology for the "green revolution". Dwarfism genes allow plants to invest less energy in vegetative development and directing more resources to fruit production. Also, shorter plants are less prone to lodging, the combined effect resulting in a dramatic increase of harvestable yield.

Similarly, fruit trees that are adapted to grow in dense populations will allow more efficient management and harvest and result in a higher yield per hectare.

Etherington *et al*. (2007) report on the effect of dwarfism genes in modifying the stature in poplar. They noted that the transgenic trees were 25% smaller than the controls in height, but had an investment in roots (vs. shoots) that was 30% greater than the controls. The preferential allocation to roots over shoots may also be the reason why an increase of stress tolerance, or recovery from poor health, has been attributed to trees treated with the GA inhibitor paclobutrazol. The semi-dwarf transgenic plants rooted at a much higher rate than wild-type plants. The mutant *gai* gene driven by the wild-type GAI promoter had the highest rooting rate.

On the contrary, traits that enhance the height of a plant, typically result in delayed flowering, *e.g.* in certain grasses. In reviewing strategies for manipulation of plant height for biomass production in maize and sorghum, Salas Fernandez *et al*. (2009) report that plant height is correlated with other traits, such as panicle length, peduncle length, grain mould (negatively correlated) and sugar yield in sweet sorghums, among others. The positive and significant correlation between plant height and lodging identified in two sorghum studies is considered agronomically undesirable.

Plant hormones/growth regulators are active in different plant parts throughout a plant's life cycle:

- BRs promote cell elongation, control tiller number, leaf size, and leaf angle, play an important role in controlling seed size and weight, and influence photosynthetic CO_2 assimilation;
- Gibberellins are involved in stem elongation, dormancy, germination transition to flowering, senescence, etc.;
- Auxins are required for axillary meristem initiation during both vegetative and inflorescence development in roots and shoots;
- Cytokinins are involved in cell division and influence seed germination, shoot/root balance, transduction of nutritional signals, leaf expansion, reproductive development, and delay of senescence.

But all are balanced by feedback and feedforward mechanisms or counteracted by the activity of other hormones.

Also signalling molecules like trehalose-6-phosphate are involved via the trehalose pathway in a wide variety of plant processes ranging from embryo and leaf development, cell division and cell wall synthesis, inflorescence architecture, seedling biomass, adult plant biomass and photosynthesis, sucrose utilization, starch metabolism, to tolerance to abiotic stresses, particularly drought.

Another phenomenon is that one abiotic stress can decrease a plant's ability to resist a second stress. Tester & Bacic (2005) indicate that low water supply can make a plant more susceptible to damage from high irradiance due to the plant's reduced ability to reoxidize NADPH and thus maintain an ability to dissipate energy delivered to the photosynthetic light-harvesting reaction centres.

5.1.2.2 "Cross-talk"

"Cross-talk" is a very specific phenomenon that should not be confused with side-effects. The term "cross-talk" refers to any instance of two signalling pathways from different stressors that

converge at a certain point (Knight & Knight, 2001). This might take the form of different pathways achieving the same end or of pathways interacting and affecting each other's outcome, including the flux through one pathway affecting another. These might act in an additive or negatively regulatory way, or might compete for a target.

Plants have evolved a wide range of mechanisms to cope with biotic and abiotic stresses (Fujita *et al*., 2006; Yoshioka & Shinozaki, 2009). To date, the molecular mechanisms that are involved in each stress response have been studied more or less independently or each other and so our understanding of convergence points between biotic and abiotic stress signalling pathways remain rudimentary. However, recent studies have revealed several molecules as promising candidates for common players that are involved in cross-talk between stress signalling pathways.

- Hormone signalling pathways govern biotic and abiotic stress responses. ABA is a phytohormone that is extensively involved in responses to abiotic stresses such as drought, low temperature, and osmotic stress. ABA also governs a variety of growth and developmental processes, including seed development, dormancy, germination, and stomatal movement. By contrast, the phytohormones salicylic acid, jasmonic acid, and ethylene play central roles in biotic stress signalling upon pathogen infection. In many cases, ABA acts as a negative regulator of disease resistance.
- Emerging evidence suggests that hormone signalling pathways regulated by abscisic acid, salicylic acid, jasmonic acid and ethylene, as well as ROS signalling pathways, play key roles in the cross-talk between biotic and abiotic stress signalling.
- The signalling molecules hexoses (fructose, glucose and sucrose) influence photosynthesis. Cross-talk of the sugar, phytochrome and light systems has been identified. Furthermore extensive interactions between sugar and plant hormone signalling is established.
- TF are the master regulators that induce or repress several pathways. When modified, the response might be balanced since all genes downstream are proportionally up- or down-regulated.
- Genes identified for yield enhancement often show to be useful in stress tolerance: *e.g.* WRKY TFs having differential roles in abiotic stress as well as in plant development; the same is true for the transcriptional co-activator STZ, HKT1 transporter protein for improved seed yield and salt stress etc.
- Some findings illustrate the modification of phytohormonal cross-talk by pathogenic microorganisms to suppress pathogen resistance response.
- Further evidence on cross-talk between biotic and abiotic stress responses is obtained from reports on environmentally sensitive *A. thaliana* mutants that show alterations in pathogen resistance response.
- There are indications of cross-talk of different signalling pathways such as the nitrogen and carbon pathway (Pakenchar *et al*., 2004) and nitrogen and cytokinin (Sakakibara *et al.*, 2006).

5.1.2.3 Unintended effects

As summarized by OGTR in DIR 071/2006 possible unintended effects may include:

- altered expression of an unrelated gene at the site of insertion;
- altered expression of an unrelated gene distant to the site of insertion, for example, due to the encoded protein of the introduced gene changing chromatin structure, affecting methylation patterns, or regulating signal transduction and transcription;
- increased metabolic burden associated with high level expression of the introduced gene;
- novel traits arising from interactions of the protein encoded by the introduced gene product with endogenous non-target molecules; and
- secondary effects arising from altered substrate or product levels in the biochemical pathway incorporating the protein encoded by the introduced gene.

Unintended effects might result in adverse outcomes such as toxicity or allergenicity; weediness, pest or disease burden; or reduced nutritional value as compared to the parent organism. All methods used for breeding or modifying plant traits, including self- and cross-pollination, the generation of hybrids or haploid breeding, mutational breeding (including X-rays or chemicals) and advanced biotechnologies (including protoplast fusion and/or recombinant DNA technology),

have the potential to generate unintended effects in plants. (Haslberger, 2003). The author mentions several examples of conventionally bred varieties and varieties developed via biotechnology; *e.g.* a conventional potato variety Lenape containing very high levels of toxic solanine. The author also stresses that environmental factors and epigenetic changes as well as genetic background influence gene expression and thus phenotypic variability; *e.g.* heat could influence variation in the expression of anti-nutrients.

Accumulated experience with genetic modification of plants indicates that, as for conventional (non-GM) breeding programs, the process has little potential for unintended outcomes that are not detected and eliminated during the early stage of selecting plants with new properties (Bradford *et al.*, 2005). Additionally, unintended changes that occur as a result of gene insertions are rarely advantageous to the plant (Kurland *et al.*, 2003).

5.1.2.4 Other approaches, same mechanisms

The requirement to conduct an ERA is limited to developments based on genetic engineering. Strauss (2003) makes the case that new approaches will increasingly be based on native or homologous genes from related species. Such genes will often modify metabolism in a manner similar to that of natural or induced mutations, but it should be possible to create desired phenotypes with greater precision and efficiency. Dominant alleles important to agricultural goals, but poorly represented in breeding populations because they are rare or deleterious to wild progenitors, can be created and inserted into varied kinds of germplasm.

Strauss (2003) argues that the improvements achieved via genomics-guided transgenes should be comparable to or of greater value than those obtained via traditional breeding approaches. Nevertheless, although a trait may be completely based on influencing native stress control pathways, the GM plant will be subject to a high level of regulatory scrutiny.

5.1.2.5 ERA for complex phenotypes

It is feasible to conduct an ERA on the basis of a complex phenotype. The starting point is that the significant differences with non-modified plants are characterized and understood. Nickson (2008) indicated that in the case of a drough-tolerance in maize possible low levels of “cross-talk” with other stress response pathways might result in a small, detectable change in response to another stress, *e.g.* heat. If this additional response is low in magnitude and inconsistent across regions, it will likely not be commercially useful. Irrespective of the commercial interest, the chacterization of such additional response(s) should be robust enough to allow determining the relevance for the environmental safety of the product.

5.1.3 Comparative assessment

EFSA (2006) confirms that the risk assessment strategy for GMOs seeks to deploy appropriate methods and approaches to compare the GMO and derived products with their non-GM counterparts. The underlying assumption of this comparative assessment approach for GM plants is that traditionally cultivated crops have gained a history of safe use for the normal consumer or animal and the environment. These crops can serve as a baseline for the environmental and food/feed safety assessment of GMOs.

Nickson (2008) confirms that the purpose of comparative product and plant characterization is to define and identify meaningful differences between the GM crop and the conventional crop. Thus, having a good understanding of the response of the conventional plant to a stressor and optimal conditions is essential as is the ability to assess interactions of the plant with other stresses.

EFSA provides detailed indications on how to set up the comparative assessment. In particular design, choice of comparators, testing environment, etc. are discussed in great detail. However, when applying these indications to a GMO harbouring stress tolerance, risk assessors will face some challenges:

- **Choice of receiving environments.** Test sites and conditions have to be selected as relevant for the possible receiving environments. It can be assumed that stress tolerant plants will be grown in the same environments as the non-modified plants. *E.g.* WUE maize is anticipated to be cultivated with a more efficient water use in the same regions as where maize was cultivated before. The trait would not result in a major extension on the cropping area in sub-optimal growing regions.

  However, if certain levels of stress tolerance are achieved, such extension in other environments might be considered. Such extensions need to be taking into account when selecting representative environments for testing and performing the comparative analysis.

- **Choice of comparator and baseline:** The choice of appropriate comparator(s) and reference varieties is essential in order to establish potential differences and to evaluate if the new material is in the available range of the crop. It is also suggested to test this under relevant conditions. In the case of a stress tolerance, this would include testing under optimal as well as stressed conditions. Under optimal conditions all material can be tested. However, when reaching a sub-optimal situation, it can be expected that the comparator(s) and the references will be effected, potentially not performing at all. *E.g.* when working with a drought tolerant plant, the modified plant as well as all other entries may be compared under normal cultivation conditions. Under drought stress, all non stress tolerant entries may be heavily impaired and not allow suitable observations being made.

  Although this poses some challenge for the design of the trials, the phenomenon is comparable to the testing of a herbicide tolerance trait. Obviously only those entries that carry a specific herbicide tolerance can be treated with the herbicide. A typical comparison would include following treatments: controls without herbicide treatment, GM plant without specific herbicide treatment and GM plant with the specific herbicide treatment. In the case of the herbicide tolerance, these entries can however be compared in a single design. In the case of stress tolerance, a test will compare controls and stress tolerant material and it will depend on the environmental conditions, if both are subject to a certain level of stress.

- **Varying stressors.** Under controlled conditions as in growth rooms and greenhouses, it may be possible to control stressors and to ensure that the plants are exposed to a single stressor. As such it may be straightforward to correlate the introduced trait with an observed performance. Under field conditions, it would be an exception that crops are exposed to single stressors. Stressor complexes may be regional as opposed to across the geographic range of the crop. Typically, control entries serve to adjust for the effect of other parameters. However, it will be important to foresee a representative selection of stressor combinations, to reflect a range of situations. This is particularly relevant if “cross-talk” or multiple phenotypic effects have been identified. Unlike a herbicide tolerant maize where the plant response and phenotype are predictable, tolerance to stress is likely to be sensitive to the presence and level of the stressor thus making stress a challenge to quantify across a wide geographic region. Nickson (2008) confirms that plant characterization studies should be conducted under conditions where the stressor situation is carefully controlled.

- **Controlled expression.** Controlled expression is a key element in avoidance of the possible growth penalty and lack of flexibility associated with continuous expression of stress defences. For the comparative analysis, it is an additional complicating factor as sufficiently different conditions would be required to cover a relevant range of expression scenarios. Expression levels may depend on the environmental situation and the presence of the stressor. For the ERA it may be more straightforward to consider a worst case scenario based on potential maximum expression levels, rather than trying to characterize in detail the levels under diverse conditions. If at the maximum level no negative effects on the environment and human health can be identified, then a more detailed analysis will not yield any more relevant information.

The above considerations are less important for traits that provide yield enhancement under optimal conditions. In order to realize their potential, optimal conditions will be required and therefore a standard comparative analysis can be performed.

5.2 Considerations in the ERA

5.2.1 Persistence in agricultural habitats and invasiveness in natural habitats

This topic covers two related but distinct potential effects of a plant, namely acting as a volunteer in agronomic systems and establishing as feral populations outside the field and so influencing biodiversity and natural populations. They are aspects of what is commonly called “weediness”. The effects can be mediated via survival and distribution of propagatable material. In most crops this is limited to seeds, although for instance also tubers and rhizomes could contribute in other crops. In this report, the focus is on seed-mediated gene flow as a mechanism for distribution.

5.2.1.1 Seed-Mediated Gene Flow

Seed-mediated gene flow (as reviewed by Warwick *et al.*, 2009 and references therein) allows transgenes to move long distances and persist over time. Seed movement includes seed transport and storage and incorporation in the soil seed bank that allows for the persistence of volunteer populations.

Depending on the proclivity of the crop to shatter, harvest seed losses to the soil surface can be high. Any trait that limits seed losses results in a reduction of seed mediated gene flow. Conversely, any modification that would result in an increased release of propagules, would increase seed mediated gene-flow. This is very unlikely in material selected for commercial introduction as it would negatively affect the harvestable yield and net return for the grower.

Seed bank inputs and seed persistence determine the recurrence of volunteer crop populations in subsequent crops. Populations of volunteers are regulated by seed predation, which is maximized where seed is at the soil surface, and by fatal germination. The initial volunteer crop seed bank is quickly reduced by seed predation, fatal germination, disease, and abiotic factors. (Warwick *et al.*, 2009)

Most of the traits involved in yield enhancement and protection are not directly related to seed characteristics. Stress tolerances are typically targeted to later stages in plant development, yield enhancement is rather oriented to plant development and production of harvestable material. A few exceptions can be mentioned and will be discussed further:
- increase on quantity of seeds;
- increase in seed size, and
- increased (or induced) seed dormancy.

Alternatively, there are also traits that will limit seed-mediated gene flow, such as inhibition of flowering to enhance biomass production and limitation of seed loss.

5.2.1.2 Weediness, fitness & invasiveness

There are no definitions or references for weediness, fitness and invasiveness in EU legislation or guidance.

- **Weediness.** A weed in a general sense is a plant that is considered to be a nuisance, and normally applied to unwanted plants in human-made settings. The term is often used to describe native or non-native plants that grow and reproduce aggressively in an undesired place. “Weediness” potential is a measure of a plant’s ability to act as a weed and is sometimes used as referring to the ability to successfully colonize an ecosystem, especially when it may also lead to the displacement of other species. However, in the latter case the term “invader” would be more suitable.

Schenkelaars (2007) pointed out that agronomists and ecologists use the terms 'weed' and 'weediness' in different ways, which is often a source of misunderstanding in relation to discussions surrounding the release of transgenic plants (Ammann *et al*., 2000). For agronomists, the problem of weediness is solved if the (aggressive) weed can be removed from the agro-ecosystem by means of chemical and/or mechanical measures. For ecologists, invasions of weeds into (semi-)natural plant communities are potentially risky, in particular as highly competitive invaders are able to disturb the species pattern and outcompete rare, *i.e.* endangered and/or protected, species. But it can be difficult to call a plant a weed, because one and the same plant species may be regarded in some parts of its area as a harmless component of natural vegetation, in others as a weed, and yet in others, even as a useful plant species.

Initial models for the predictions of weediness and invasiveness were simple correlations between plant traits and known weeds. Baker (1965, 1974, 1991) produced a list of 13 plant characteristics that favoured weediness, including the following:

- discontinuous germination and long-lived seeds;
- rapid seedling growth;
- rapid growth to reproductive stage;
- long continuous seed production;
- self-compatible, but not obligatorily self-pollinated or apomictic;
- if outcrossing, uses wind or unspecialized pollinator;
- high seed output under favourable conditions;
- germination and seed production under a wide range of environmental conditions;
- high tolerance or plasticity of climatic and edaphic variation;
- special adaptations for dispersal;
- good competitiveness achieved through, for example, allelochemicals or choking growth; and
- if perennial, then with vigourous vegetative reproduction, brittleness at the lower nodes or of rhizomes or rootstocks, and ability to regenerate from severed rootstocks.

Weedy species were considered to have more of these traits than non-weedy species. Although Baker's characteristics have been criticized, his weedy trait list and definition of an "ideal" weed are still generally accepted as the key adaptive characteristics contributing to the success of a plant as a weed (Williamson, 1994) and there is no formulation that is clearly superior at this time. Nevertheless, the quantification of the characteristics would be most helpful.

Domestication has generally reduced weedy and invasive tendencies of the crop species. Although the degree of domestication varies by crop, many of the current GM crops share a similar suite of domestication traits (Warwick & Stewart, 2005). Despite the great diversity of genes, many of the modified traits are familiar, having a long history of domestication and consequent reduced fitness through artificial selection. Male sterility, seedless fruits, delayed spoilage, and dwarf stature are familiar examples.

Domestication traits are frequently the inverse of weediness traits. Many abiotic and biotic factors limit the ability of crops and hybrids to form self-sustaining populations under either cultivated or uncultivated conditions. Traits that confer tolerance to biotic and abiotic stress will probably increase the survivorship, biomass, and fecundity of crop volunteers and crop-wild/weedy hybrids under stressful conditions. However, an increase in fecundity is not necessarily a good predictor of population expansion or invasiveness (Bergelson, 1994; Cummings & Alexander, 2002).

An important element in predicting weediness is taxonomic relationship, considering weediness within a taxon, including its history of weediness in any part of the world.

Warwick *et al.* (2009) argue that predictions of the ecological impact of single or stacked stress tolerance traits in host/target environments may be aided by an examination of stress tolerance traits exhibited in select model annual weed species. Kochia could serve as a

model species in that it possesses extensive herbicide, insect, and disease resistance; high NUE; and drought, heat, cold, and salinity tolerance (Friesen *et al.,* 2009).With one of the highest rates of increased relative abundance and spread among North American weeds, its success is undoubtedly a consequence of the interactive effects of these multiple traits.

Overall, there are relatively few data available with which to evaluate the potential for increased weediness or invasiveness in a crop species with fitness-enhancing abiotic and biotic GM traits. A better understanding is needed of the factors that presently control population size and range limits of either the crop volunteers or wild recipient populations, and the degree by which survival or reproduction in the field is presently affected by the relevant biotic or abiotic stress tolerance trait.

- **Fitness.** Orr (2009) defines "fitness" as the capability of an individual of a certain genotype to reproduce. In genetic models fitness is usually equal to the number of allele copies that an individual contributes to the next generation. Absolute fitness of a genotype is defined as the number of alleles of type a1 passed on. Relative fitness of an alternative allele a2 is quantified as the number of a2 copies passed on relative to the number of a1 copies passed on.

  If everything else is the same it is convenient to say that an allele that increases surival or seed production has a higher fitness, *i.e*. it will increase in frequency in the population. When alleles affect multiple characters it is not obvious which allele wins in the course of natural selection. An allele might, for instance, have a negative effect on seed production (-) but may also result in faster reproduction, *i.e.* in a smaller chance of dying before the end of life (+). When genetic effects are complex these effects are often combined in a single measure that corresponds to "fitness".

  A composite "fitness" measure of relative plant germination, emergence, growth, and fecundity under intra- and interspecific competition, is the finite rate of increase (λ), a composite measure of fitness. This is an appropriate measure of fitness under a wide range of conditions (Parker & Kareiva; 1996) because it compounds measures across all stages of the plant's life cycle and incorporates the speed of reproduction. However, alternative definitions are also possible. For instance, several authors have argued that under some conditions expected reproductive success, R_0 (how much offspring an individual is expected to make during its life, regardless of the time that this takes), is the correct proxy of fitness (de Jong & Klinkhamer; 2005).

  Fitness levels can vary with the environment, and both controlled and field experiments should simulate selection under agricultural/ecological conditions (*e.g.*, costs are generally more obvious in controlled environments and harder to detect under more severe abiotic and biotic conditions in the field [Parker & Kareiva, 1996]).

  Rate of spread of an allele (including transgenic alleles) is governed mainly by its effect on fitness rather than by migration rate (Rieseberg & Burke, 2001; Burke, 2004; Chapman & Burke, 2006). If genes would release a population from a major limiting factor, even rare transgene escapes could become established and begin to spread across the range of the recipient species. On the other hand, if cross-pollination is very frequent, but the gene does not add to the fitness of the resulting hybrid, it will not spread. Theoretical studies (Haygood *et al*., 2004) revealed that even low hybridization rates (on the order of 10^{-3}) might allow for the rapid distribution and establishment of a moderately favourable transgene. Transgenes could have a significant ecological advantage if they increase the recipient's interspecific competitiveness, increase its ability to invade an expanded niche range, or precipitate a severe decline in herbivores or plant pathogens that limit its growth (Weis, 2005).

  However, not all such changes would necessarily promote weediness/invasiveness. For example, a gene that increases interspecific competitive ability could induce a genetic sweep through the recipient population without changing its abundance and density relative to the larger community, although the genetic diversity and structure could be affected (Weis,

2005). Thus, predictions on the implications of transgene spread require some knowledge of the demography and genetic structure of the recipient populations, how they interact with other species, and what factors limit their spread.

Furthermore, traits advantageous in agricultural habitats may not have similar benefits in natural areas. Gene expression analysis on wild and weedy *Helianthus annuus* L. suggested that wild populations, adapted to open grasslands, express genes associated with biotic and abiotic stress, whereas weedy *H. annuus*, adapted to agricultural fields, have down-regulated expression of stress tolerance genes (Lai *et al*., 2008). Seedlings of the weedy *H. annuus* grow twice as fast as those of the wild biotypes. The authors hypothesized that there is an energetic cost associated with stress tolerance and that weedy species have adapted to agricultural conditions by down-regulating stress tolerance adaptations, allowing for an increase in seedling growth rate.

The general belief is that traits conferring resistance in plants have a fitness cost. However, a 1996 survey of 88 published comparisons of tradeoffs between resistance and fitness traits in plants (Bergelson & Purrington, 1996) found that costs were most often associated with resistance to herbicides, followed by resistance to pathogens, and least often associated with herbivores. Costs were more often found in crops than wild species. There was a large variation in the cost associated with a given resistance trait in different genetic backgrounds. The net effect of benefits and costs of the trait can be quantified by species fitness within environments.

Few studies have assessed reproductive fitness of GM crop volunteers or GM crop × weed hybrids for putative fitness-enhancing transgenes (Burke & Rieseberg, 2003; Mason *et al*., 2003; Snow *et al*., 2003; Halfhill *et al*., 2005). Most field trial data generated to date show no or limited instances where GM crops and hybrids had an increase in fitness. Few generalizations can be made about characteristics of invading species and the target/host communities, making it difficult to predict the impact of abiotic and biotic stress tolerance traits. Much speculation exists as to what combination of traits, GM or other, it would take to enhance weediness and invasiveness.

Wilkinson & Tepfer (2009) discuss the relevance of the use of fitness parameters in decision making on GMOs. They suggest a tiered approach and remind that higher tiered testing like performed by Crawley *et al.* (1993) as part of a larger research project is protracted and demanding of space to have practical utility for early-stage screening. They indicate that such consideration is particularly relevant for stress tolerant plants that could exhibit advantageous characteristics across a range of settings outside the farmed environment, giving rise to ecological release.

Traits that improve abiotic stress tolerance of crops, including tolerance of cold, heat, salt, and drought, would appear to pose a higher risk of spread in the environment than domestication traits. Several reports (*e.g.* Ellstrand & Hoffman, 1990; Ellstrand, 2001) mention that GM traits that enhance abiotic stress tolerance have a great potential to enhance fitness. Crops with these traits may be widely planted and can occupy larger niches, including marginal and even novel environments not used by current domestic crops.

Strauss (2003) reports that despite intensive direct and indirect traditional breeding for abiotic and biotic stress tolerance in annual crops, where populations or species adapted to highly diverse ecological conditions are hybridized, inbred, and effectively cloned, there appear to be no known cases where populations that are substantially more invasive in the wild were generated as a consequence. The author further notes that it appears that wild plants achieve stress resistance differently from crops bred for high yield under agricultural conditions.

Natural adaptations to highly stressful environments often involve multiple physiological mechanisms controlled by sets of elaborately regulated genes. Modification based on one or

a few genes to promote stress tolerance in agronomic environments may therefore not significantly elevate fitness in wild plants and could even do the opposite.

This last observation is based on a report by Chen & Murata (2002) who studied transgenic plants that accumulated various compatible solutes in relation to stress tolerance. They point out that several types of compatible solute approaches may be of only limited utility because of the detrimental effects associated with high concentrations of these compounds.

Organismal regulatory systems are expected to be under strong stabilizing selection due to natural selection and their high degree of internal complexity (Siegal & Bergmann, 2002). Strong modifications to such systems are therefore likely to be deleterious to fitness in wild environments.

- **Invasiveness.** For a species to be invasive, it must pass several filters successfully. It has to be transported and released into the new environment; it should be able to survive and reproduce and then may spread, being present in high or low numbers (Colautti & Mac Isaac, 2004). This means that individual populations are evaluated and not entire species, in relation to a specific environment.

  Wilkinson & Tepfer (2009) point out that stress tolerances have been associated with selection in natural habitats based on publications by Eveno *et al.*, 2008; Franks *et al.*, 2008; Kane & Rieseberg, 2007; and Roelofs *et al.*, 2008. These papers indicate that when comparing in certain species plants that are adapted to non-cultivated, marginal conditions show specific stress tolerances. This is probably not a very astonishing finding given that in the non-agronomic ecosystem more stress factors will act continuously. While demonstrating that natural selection favours plants with inherent stress tolerance in certain stress-rich conditions seems logic, the reverse, namely that the presence of a stress tolerance likely results in a plant that gives rise to ecological release, cannot be concluded on this basis.

5.2.1.3 Considerations for specific traits

- **Cold tolerance.** Cold tolerance at early growth stages is an ecologically important trait that would allow for earlier germination and a jump-start on use of available resources, increasing the effective growing season and reducing the risk of frost damage. Fitness benefits and costs of cold tolerance have been extensively studied in *A. thaliana* (Jackson *et al.,* 2004; Korves & Bergelson, 2004). Costs were detected in the presence of greater intraspecific competition (Korves & Bergelson, 2004). A comparison by Jackson *et al.* (2004) of three cold tolerance genes (CBF1, -2, and -3 from the CBF [C-repeat/dehydration responsive element binding factor] cold tolerance pathway) in *A. thaliana* in various temperature environments by using multiple insertion lines for each transgene indicated that costs of cold tolerance, as determined by fruit number, varied by individual transgene. CBF2 and -3 over-expressers showed costs of cold tolerance and no fitness benefits in both the cold and control environments, whereas CBF1- over-expressing plants showed no fitness cost of cold tolerance in the control environment and showed a marginal fitness benefit in the cold environment. Thus, constitutive expression of traits that are normally induced in response to environmental stress will not always lead to costs or benefits without that stress.

- **Nitrogen Use Efficiency.** Soil nitrogen availability can alter crop - weed competitive interactions, depending upon plant species and densities (Carlson & Hill, 1985; Ampong-Nyarko & deDatta, 1993). Thus, species that use soil-available nitrogen efficiently may have a competitive advantage under soil nitrogen conditions limiting to plant growth. Small-plot trials conducted in Minnesota in 2003 and North Dakota in 2004 indicated greater seed yield of GM-NUE *B. napus* canola than with the control (non-GM) cultivar under various soil nitrogen levels (Strange *et al.*, 2008).

  Weed species vary greatly in their NUE. Although the NUE of weed species affects crop competitiveness, there is little information on how it may affect invasiveness. Invasive exotic species are thought to be successful invaders partially because of enhanced resource use efficiency (Vitousek, 1990; Funk & Vitousek, 2007).

- **Drought Tolerance.** The drought tolerance trait may increase yield and reduce the use of water by agriculture. Two of 24 GM drought tolerant wheat lines yielded 20% greater than the non-GM control under drought conditions in field trials in Australia (Spangenberg, 2008). Water use efficiency, transpiration rate, and response to declining soil water availability and water stress vary widely among weeds and crops, influencing the outcome of weed - crop competition (Radosevich & Holt, 1984). Few weed species show extreme drought tolerance (Forcella, 1985).

- **Salinity Tolerance.** Although many halophytic species can thrive under soil salinity conditions that are adverse to crop growth (Braidek *et al.*, 1984), not many weed species are saline tolerant (Forcella, 1985). Acquisition of this trait, similar to drought tolerance, could potentially expand the typical range of a wild relative, not unlike the introduction of this trait in the crop. Salinity tolerance could enable GM crop volunteers and crop x weed hybrids to colonize, reproduce, and spread in relatively bare saline soil where emergence and growth of many other plant species are inhibited.

  Several studies have examined salinity tolerance in roadside weed populations. Rothfels *et al.* (2002) found that field populations of the invasive weed species dame's rocket (*Hesperis matronalis* L.) were more likely to survive salinity treatments than roadside populations. In contrast, DiTommaso (2004) found that seeds from roadside populations of common ragweed (*Ambrosia artemisiifolia* L.) exhibited consistently greater total germination and rate of germination at higher salinity concentrations than seeds from agricultural populations. Selection in ragweed populations was probably enhanced by its larger populations and the imposition of very strong selection pressure over many years. Early spring emergence along roadsides provides a competitive advantage. This tolerance in germination to elevated salinity levels is probably a major reason for the dominance and invasive spread of this species along major roadways of eastern North America. Acquisition of salinity tolerance might enhance the feral nature of GM crop volunteers, especially in *B. napus*, which is already known as a feral weed of roadside habitats (Knispel *et al.,* 2008).

- **Yield enhancement traits.** At a first glance, many of the yield enhancement traits show similarity with characteristics included in Baker's list, as exemplified by the following examples:
  - induced dormancy may result in build-up of viable seed bank in the soil;
  - rapid establishment and growth could be of interest for biomass production;
  - rapid growth and earlier transition to the reproductive stage may shorten the generation time or may extend the seed filling phase;
  - taller plants may be better competitors for sunlight;
  - efficient competitors for nutrients will leave less resources for other plants,
  - an increased, continuous seed production will result in high seed output under favourable conditions; and
  - bigger seeds (although there have been reports that bigger seeds may yield more robust and better competing seedlings, there are also indications that predators such as birds preferentially feed on larger seeds).

  On the other hand, there are also yield enhancing traits that are clearly in line with further domestication, such as:
  - reduction of dormancy and improved synchronization of germination;
  - dwarfism and repression of shade avoidance limit the ability of a plant to compete for light;
  - limiting of branching and vegetative production; and
  - reduced dispersal of seeds *e.g.* by avoiding loss at harvest.

  As pointed out the introduction of a trait that is also present in some weeds should not be interpreted as transforming that crop species into a weed. *E.g.* modifying a maize plant to produce twice as much seeds or seeds of twice the seed size of control maize while leaving all other phenotypic parameters unchanged, would not result in an increased weediness

potential that would lead to impacts as a volunteers or as invasive plants. Obviously the accumulation of several modifications would be required in order to enhance the weediness potential of such crop. As highlighted before this is also an essential part of the evaluations conducted by the US and Australian authorities: given the specific of the species, the receiving environment and the introduced trait, it was deemed that no significant influence on the weediness characteristics were to be expected.

While this approach is valid in early, isolated cases, it will be of interest to anticipate that in the future more strategies will be combined in selected crops. It is at this moment unclear which of these combinations could eventually lead to a significant change in behaviour of the plants in this respect. Furthermore, there are some crops which seem less domesticated. Oilseed rape is an example. Although a poor performer in non-agronomic ecosystems, it already has a number of characteristics that are common with weeds. In such a case, special attention may be required when adding a new trait that could reflect an additional weediness potential. Conversely, traits such a reduction of pod shattering would be seen as reducing the weediness potential.

5.2.2 Selective (dis)advantages

All the traits described in this report are oriented to enhancing the performance of crop plants (including trees) under specific agronomic conditions. The advantages are in a first instance oriented to obtain more and better products or to secure products under sub-optimal conditions.

As pointed out in the previous section, some traits may provide a competitive advantage whereas others (*e.g.* those that enhance domestication) may result in a competitive disadvantage when exposed in non-managed ecosystems.

Abiotic stress tolerances elicit an expectation of competitive advantage. However, a real advantage for the modified plant may actually be very limited or not even existing. *E.g.* when considering WUE it has to be taken into account that the plants will still require water. The available water will be used more efficiently, but below a certain level of water the modified plants will also not survive. This already limits the potential for changed competitive behaviour of the WUE plant to non-managed areas that have water availability above the threshold levels.

Subsequently, there are several other factors, including biotic and abiotic stressors that influence competition in these environments. The natural population that is endemic has evolved over time and is fully adapted to the conditions. Providing a single competitive trait that may make a crop species more suited for a certain habitat; does not automatically result in a competitive advantage over the native flora. Furthermore any advantage would need to be balanced against the fact that tolerance traits usually come with a fitness cost. In some cases the negative impact on the plant is potentially so high, that tightly controlled promoter sequences are required to limit the expression. Inevitably this leads to the need for a case-by-case assessment. A similar reasoning can be developed for traits affecting plant architecture.

Wilkisnon & Tepfer (2009) offer the tiered approach to risk assessment as a practical solution:

- Tier 1 assessments can be designed on the basis of crude exposure. *E.g.* In the case of salt tolerance, simple exposure tests could be designed so that the progression to Tier 2 tests could be based on the ability of transgenic plants to survive salt concentrations set some level below that observed in natural saline-dominated communities. Similarly, Tier 1 tests for drought tolerant GM plants would assess the ability to survive maximum water availability in the arid-dominated habitats under consideration. The underlying reasoning being that if the transgenic plants cannot survive the levels of abiotic stress experienced by native plants in these communities, then they will be unable to invade them. However, if the GM plants are able to survive in these conditions of average stress, it would be important to also assess their ability to survive under conditions that would correspond to exceptional conditions (*e.g.* extreme drought), since these may be particularly important for determining long-term survival in the target environment.
- Tier 2 experiments are rather more challenging, given the complexity and heterogeneity of natural communities. Here again, however, it must be remembered that the primary goal of

the exercise is to aid decision-making rather than to perform a truly predictive ecological study. Arguably the simplest approach would be to conduct a life history comparison between transgenic and conventional equivalents under growing conditions that simulate those of the natural community under consideration. If the transgenic plants perform no better than the conventional plants, then they are unlikely to be more invasive. Similarly, if the transgenic plants are predicted to progress towards extinction, then they are also unlikely to invade, provided that numbers of founder populations (spillage rates) are not expected to be raised and population decay rates are not projected to be slow (λ is only slightly below 1).

- Progression beyond Tier 2 would necessitate a simulation of the natural community or transplant experiments of the type developed by Crawley *et al*. (1993).

As pointed out by Schenkelaars (2007), "cross-talk" may result in modifications in different stress responses, thereby providing different competitive advantages. Nickson (2008) suggests that in the case of drought tolerant maize, a small, detectable change in response to another stress, *e.g.* heat, could be attributed to "cross-talk". However, this change was insufficient to be of agronomic importance. The importance of product characterization as the first step of the ERA has been highlighted. Documenting the effects of "cross-talk" mechanisms and side-effects are a critical part of characterization.

Wolt *et al*. (2009) foresee that problem definition for ERA will identify those scenarios that merit detailed risk characterization. Whenever an effect due to cross-talk can be anticipated and this effect is deemed significant, it can be included in the ERA. Nevertheless, the first step will be to assess if the postulated impact is significant based on the expected exposure and environmental context.

5.2.3 Gene transfer to the same or other sexually compatible plant species

Gene flow from GM crops to non-GM or other GM crops and to weedy or wild relatives has been extensively investigated during the past decade. Gene flow is defined as the change in gene frequency in a population because of movement of gametes, individuals, or groups of individuals from one location to another (Slatkin, 1987). Such movement can occur via pollen, seeds, or vegetative propagules, the relative importance of which varies according to plant species.

5.2.3.1 Pollen-Mediated Gene Flow in Genetically Modified Crops

Many biological, environmental, and crop management factors influence the frequency and distance of pollen flow between GM donor and non-GM or other GM fields. Such factors include type of vector (wind and/or insect), genotype or cultivar, fertility (*e.g.*, male fertile or -sterile receptor plants), pollen viability and longevity, synchrony of flowering or pollen production, wind speed and direction, air turbulence/convective air currents, temperature, humidity, and relative density of donor and receptor plant populations (Beckie & Hall, 2008). At a landscape scale, pollen flow can also be affected by topography; vegetation; distribution and abundance of volunteer and feral (self-perpetuating) populations; and number, shape, and spatial arrangement of pollen donor and receptor fields.

Most of the traits discussed in this report are not expected to influence intra-specific pollen-mediated gene flow. It can be anticipated that a GM plant that produces more flowers, may produce more pollen and that subsequently the relative presence of GM pollen would be more important. Nevertheless, this would not create a new issue or change the mechanism by which pollen-mediated gene transfer occurs. On the contrary, in cases where flowering is delayed, less GM pollen would be released compared to conventional varieties. Overall, this quantitative aspect needs to be projected against the deployment of GM varieties compare to non-GM varieties.

Schenkelaars (2007) cautions that as a drought tolerance trait in a drought tolerant GM maize could potentially affect the metabolism of its pollen, it cannot be excluded that this might change the viability of its pollen, and consequently, the dispersal characteristics of its pollen. However, while theoretically of interest, there are no indications that such an effect on pollen metabolism will occur. The need to control the expression of stress tolerance traits in order to avoid

deleterious effects on plant development has been clearly demonstrated. Even if a stress tolerance trait that is relevant during the vegetative or reproductive life cycle phase is expressed in pollen, there is no indication that it will also affect the stress tolerance of pollen.

5.2.3.2 Interspecific Gene Flow between Genetically Modified Crops and Wild Relatives

Crop-wild hybridization is taxonomically and geographically widespread (Ellstrand *et al.,* 1999; Poppy & Wilkinson, 2005) and is certainly not unique to GM crops. Interspecific hybridization frequencies and evidence for introgressive hybridization have been documented in many crop-wild species complexes, with 22 of the world's most important crops showing evidence of hybridization with at least one wild relative (Ellstrand *et al.,* 1999; Warwick & Stewart, 2005).

Various factors will affect interspecific hybridization frequency between GM crops and wild relatives (reviewed in Chèvre *et al.,* 2004; Mallory-Smith & Zapiola, 2008), including degree of compatibility, spatial isolation of crop and wild populations, relative density of the recipient wild population compared with that of the crop source, synchrony of flowering, direction of the cross, parental genotypes, and presence of pollen vectors. Introgression of genes into populations of a wild species, that is, the incorporation of genes from one differentiated gene pool into another, will occur only if barriers of incompatibility, genetic instability, and low hybrid pollen fertility are overcome. Stable introgression through the formation of backcross (BC) generations is also dependent on F1 hybrid fitness, that is, their growth vigour, fertility, ability to set viable seed, and persistence in the seed bank. The progeny of interspecific crosses often exhibit reduced fitness, but the fitness of crop × wild hybrids has been found to vary greatly across taxa and might also vary widely across different environments and in different seasons.

Mercer *et al.* (2007) report on conditions and characteristics that may promote introgression. We compared diverse crop-wild hybrid genotypes relative to wild *Helianthus annuus* under one benign and three stressful agricultural environments. Although 'domestication' traits are typically considered unlikely to persist in wild populations, they indicate finding some (*e.g.* rapid growth and early flowering) that may enhance hybrid fitness, especially in stressful environments.

Stress tolerance traits may extend the range of a transgenic crop, permitting its growth in regions adjacent to a wild relative from which it was previously geographically isolated. This expansion may facilitate natural hybridization between distinct but compatible species that had never previously hybridized (Grant, 1981).

Given the propensity for crop-wild hybridization, one should assume that some hybridization is possible even if hybridization frequencies are low. Several authors have advocated shifting the focus from the overall rate of hybridization to fitness effects of the gene(s), that is, the selective advantage of the transgene.

Lu (2008) points out that GM crop volunteers or hybrids between a GM crop and wild relatives have the ability to become more effective and aggressive weeds, after incorporating transgenes that convey traits against biotic and abiotic stresses. This is based on the hypothesis that a transgene from a GM crop will bring a fitness advantage to the populations of crop volunteers, weeds and wild species (Lu and Snow, 2005). Depending on local environmental conditions, the flow of these types of "fitness-enhancing" transgenes to nearby recipients could release weedy or wild populations from ecological pressure that restrict their local abundance and limit their habitat requirements.

Lu & Snow (2005) elaborate that several interrelated questions arise regarding the environmental effects of fitness-enhancing transgenes in rice. Given these genes' potential to spread and persist, their possible negative effects on non-target species should be considered. For a given country and region, it is important to ask whether the receiving population is already a weed or has the potential to become more invasive by acquiring specific transgenic traits. The consequences of gene flow from transgenic rice relates to the magnitude of the fitness benefit from transgenes, and whether this benefit affects population dynamics, should be addressed. Virtually nothing is known about the extent to which insect and disease pressures regulate populations of weedy or wild rice, and ecological studies of these populations are needed to help

address this question. Likewise, nothing is known about how novel genes for increased crop yield will affect the growth and fecundity of weedy or wild rice.

Snow *et al*. (2005) further elaborate on the fact that if fitness-enhancing transgenes become established in natural populations, the populations may or may not become larger, more widespread, or more difficult to manage, depending on ecological factors that limit population growth. Over the longer term, certain weeds may benefit from transgenes that confer faster growth and resistance to herbivores, diseases, or harsh growing conditions. Initially, the effects of one or a few transgenes may be difficult to detect unless weed populations are released from strongly limiting factors (*e.g.*, drought stress or salinity). For most weeds, little is known about the extent to which various ecological factors limit the weed's abundance, competitive ability, or geographic range. These data gaps present challenges for predicting whether transgenic weeds could become more difficult to manage than those that lack novel transgenes.

5.2.4 Interactions with target organisms

There are no reasons to assume that a yield enhancing or yield securing trait will have a direct effect on herbivores, parasitoids, and pathogens. Firstly, because these traits are not aimed to control pests at all, and therefore no target organisms can be defined. Secondly, as neither the genes and their products, like plant signalling factors or enzymes, nor the metabolites of these enzymes are known to have an effect on herbivores, parasitoids, and pathogens. Indeed, the genes are not meant to interfere with secondary metabolism. However, it might be that carbon/nitrogen ratios are changed due to NUE. On the other hand, because of the tight coupling of carbon and nitrogen assimilation, changes are expected to be small.

It is recognized that due to "cross-talk", some abiotic stress response mechanisms are shared with biotic stress signalling pathways. Examples are the hormone signalling pathways and MAP-kinase cascades, *e.g.* AtMYC2 and AtMYB2 (Abe *et al*., 200). AtMYC2 is a TF inducing drought response via ABA and in many cases, ABA acts as a negative regulator of disease resistance. AtMYC2 over-expressers could be more sensitive to pathogen infections. In plants, the MAPK cascade plays a crucial role in various biotic and abiotic stress responses and in hormone responses that include ROS signalling (Fujita *et al*., 2006 and references therein). Nevertheless, these responses are a reaction of the plant to the stressor and are not targeted in a real sense against the stressor, biotic or abiotic.

5.2.5 Interactions with non-target organisms

According to the definition proposed by EFSA (2010), potential non-target organisms (NTOs) are defined as all those species directly and/or indirectly exposed to the GM plants, and which are not targets of the newly expressed metabolite(s) in these plants. So far, the intended traits have a role in protecting the plant against the effect of a stressor. Nevertheless, this protection is not aimed at affecting the stressor. For the cases observed until now, it can be argued that also NTOs will not be affected. Even if a plant responds to a disease due to "cross-talk" induced by an abiotic stressor, the disease may locally become less effective, but is most likely not affected at the ecosystem level. Although on this basis no negative effects can be expected, it is probably too early to make a general conclusion and a case-by-case approach should be maintained. This allows to evaluate each specific approach and to confirm that no effect on non-target organisms will occur.

5.2.6 Effects on human health resulting from potential contact

Addressing food and feed safety is outside of the scope of this report. Nevertheless, effects resulting from contact with the GM plant and potentially unintended consumption could be an issue. Even more so than looking at environmental effects, this is based on a case-by-case approach, focusing on the specific protein(s), metabolic change(s) and metabolites. Many of the strategies that are deployed are derived from plants and/or are conserved in various plants that have a history of safe use.

Cassels & Doyle (2003) point out that some stress metabolites, *e.g.* phytoalexins, and stress proteins, *e.g.* chitinase used to improve biotic resistance, may be anti-nutritional and allergenic respectively. This poses a potential risk to consumers where these are used as the basis of tolerance or where their expression is increased due to the presence of transgenes.

Another possible scenario includes that by protecting a plant against a stressor, other stress responses or metabolic pathways that are native to plant produce undesired compounds. Under normal conditions these would not become available as the plant may not survive the stress factor. Some examples:

- GM tobacco contained a higher level of nicotine and lower levels of anabasine indicating that the metabolic pathways had changed (Holmberg *et al*., 1997).
- When *Datura innoxia* was transformed with *Vitreoscilla* haemoglobin, a six-fold increase in the levels of the alkaloid scopolamine could be observed over the controls (Bülow *et al*., 1999).
- OGTR addressed the issue for gossypol and cyclopropenoid fatty acids in nsHbs cotton: (DIR067 and DIR083).
- Another example is the ornamental torenia (DIR084/2008) modified to enhance phosphate uptake. Due to the action of the inserted gene some metabolic adjustments took place which increased secondary metabolism leading to enhanced indole alkaloid production (OGTR, 2008e). If this would be a food plant, further investigation would be legitimate.
- Park *et al*. (2005) warn for a possible deleterious consequence of over-expression the AVP1 proton pump in tomato. It could be that toxic metals accumulate in the fruit of transgenic plants. However, evaluating fruit cation contents of control and GM tomatoes showed no significant difference in levels of Pb^{2+}, Mo^{2+}, Mn^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} or Ca^{2+}.

Based on the fact that most of the genes that are deployed are obtained from plants or from other organisms with homologues or orthologues in plants, it can be anticipated that although there may be changes in the levels of metabolites produced as a result of the activity of the encoded proteins, these metabolites are most likely known and are not new to the plant. The risk assessment approaches that are in place are expected to adequately address associated concerns. Developers include early screening for a potential relationship with an anti-nutritional, toxic or allergenic characteristic in their projects. With an increasing understanding on the relationships between the different pathways, a thorough identification can occur of potentially influenced products. Finally, when complex changes are envisaged, feeding trials are required to demonstrate safety for man and animals.

In case Food & Feed approval is aimed for, the guidance documents from EFSA (2006) describe explicitly the requirements for GM plants with extensive intended genetic modifications. EFSA indicates among the examples GM plants which have been extensively modified in order to cope with environmental stress conditions like drought or high salt conditions. It is expected that through insertion of multiple genes or gene cassettes the internal metabolism in these GM plants may have changed significantly, leading to profound compositional alterations which may have an impact on the health or nutritional status of the consumer. Moreover, besides intended alterations in the composition, unintended and unpredicted changes may take place, which may not always be detected by the usual compositional analyses of major macro and micro nutrients, or naturally occurring toxins, and which may impact on human/animal health or nutritional status. In this case, the testing program shall include at least a 90-day feeding study in rodents.

5.2.7 Effects on animal health and animal feed

There are no ex-ante reasons to assume that the use of crops with yield enhancement or yield preservation traits as animal feed will have detrimental effects on animal health. Nevertheless, this must be based on a case-by-case approach, focusing on the specific protein(s), metabolic change(s) and metabolites.

5.2.8 Effects on biogeochemical processes

Schenkelaars (2007) concludes that there are no reasons to assume that incorporation into the soil of root exudates, plant litter, seeds or pollen of a drought tolerant GM maize will have effects

on biogeochemical cycles. The conclusion is largely based on the very low likelihood of horizontal gene transfer and absence of expression of genes under the control of eukaryotic promoters with very limited, if any, activity in prokaryotic organisms. It is also based on the fact that neither the drought tolerance genes and their products, nor the metabolites of these enzymes are known to have effects on soil microbes. However, this may be different for plants with changed metabolic pathways which can affect other pathways by 'cross-talk'.

There are no indications that this would be different for most of the other strategies that are deployed for yield enhancement and yield preservation. There are however two specific cases that may require specific attention:

- Whenever an abiotic stress response results in increased levels of root exudates to make nutrients available, it will also influence rhizosphere micro-organisms.
- Enhanced uptake of nutrients from the soil could deplete the soil and "starve" surrounding vegetation.

In discussing the potential impact of transgenic crops on soil nutrient transformations, Motavalli *et al.* (2004) address transgenic crops with improved traits to acquire nutrients from soil. Examples of targeted traits that have been researched include improved plant tolerance to low Fe availability in alkaline soils, enhanced acquisition of soil inorganic and organic phosphate, and increased assimilation of soil nitrogen. Improvements in plant nutrient acquisition have also been observed in transgenic crops, which have traits for overcoming other abiotic stresses, such as soil salinity and drought tolerance. Removal of plant growth limitations may be expected to increase plant utilization of soil nutrients since nutrient demand be higher in healthier plants, but additional changes in plant physiological characteristics due to the introduction of novel traits may also affect nutrient acquisition.

Motavalli *et al.* (2004) point out that the mechanisms by which these transgenic crops may overcome nutrient deficiencies, such as increased root exudation of organic acids, may directly or indirectly affect soil microbially mediated nutrient transformations. For example, the decrease in rhizosphere soil pH due to increased root exudation of organic acids may reduce the rate of microbially mediated processes sensitive to acidic pH (*e.g.*, nitrification). However, the presence of organic anions in soil can also stimulate microbial activity and increase nutrient availability.

Mimura *et al.* (2008) report on an investigation of the potential impacts of environmental stress-tolerant crop production on soil microbial communities and soil functions in saline and non-saline conditions. The results obtained with potato transformed with *DREB1A* suggest that abiotic growth environments had a stronger impact on soil microorganisms and biochemistry than did the plant genotypes. This confirmed their initial position that although changes in root exudation of plants may affect the structure and functional diversity of soil micro-organisms; soil types, abiotic conditions, plant types and plant physiological stage also influence microbial communities.

5.2.9 Specific cultivation, management and harvesting techniques

It is not expected that the crops modified with yield enhancement or yield preservation traits will require a change in cultivation, management and harvesting techniques.

Most authors point out that a major impact can occur when the traits would lead to introduction of the crop on areas, *e.g.* on marginal land, where until then cultivation was not possible. There is however little information available that would support making clear predictions on this subject.

EFSA (2010) specifies that the receiving environment will generally include the environment where the specific plant (species) has already been cultivated, but may also include areas where the new traits will allow cultivation outside of former cultivation areas (*e.g.* for GM plants with tolerance to abiotic and biotic environmental stresses or providing new economic benefits).

Nickson (2008) suggests that based on the product concept, the problem formulation might consider whether the trait could expand the range in which the plant will be cultivated or could grow. *E.g.* in the case of drought tolerant maize, the applicant indicated that the product is

intended to be grown and used as any other maize in the same area with same practices, only that it will be less susceptible to low water provision.

5.3 Conclusions for the ERA

Following an overview of techniques that are deployed for engineering yield enhancement and yield preservation in plants, this section addressed the concerns that have been raised in relation to the ERA for these traits. Indeed several authors refer to these traits as requiring special care when performing an ERA, as they are "felt" to possibly lead to important environmental effects. In accordance with the structure proposed in the Annex II.D.2 of the European Directive 2001/18/EC, the issues were systematically reviewed focusing on those elements that would be unique and/or novel for yield enhancing or preserving traits.

As this review indicates, there are some features which necessitate that the ERA needs to take other aspects into account than for *e.g.* an agronomic trait like herbicide tolerance or insect resistance:

- Complex phenotypic effects induced by response mechanisms, including the phenomenon of "cross-talk", inherent to the plant may, require adapted designs for product characterization. Fully characterizing all significant changes that are relevant for the ERA may be more challenging than for other agronomic traits;

- The importance of different environmental conditions as possibly prevailing in the receiving environment has to be taken into account when planning the comparative analysis;

- Although the traits provide a specific competitive behaviour to the modified plants, it is highly unlikely that the modification of one trait, *e.g.* a single tolerance to a particular abiotic stress, will turn a crop plant into a weed. Nevertheless, due to a lack of our understanding of the environmental conditions that control species, it remains impossible to predict which combination of traits will result in an ecological release. Possible advantages of stress tolerance are often balanced with costs associated with the expression of the functions. In order to limit the negative impact on the plant's performance, the expression needs to be controlled by regulatory sequences;

- Although in some cases it can be argued that improved fitness may enhance the rate of introgression of the trait in related species via pollen-mediated gene flow, once introgressed the impact of traits is expected to be minimal;

- Seed-mediated gene flow will in most of the cases not be influenced. No generic effects are anticipated on target organisms, non-target organisms or other plant associated organisms, on human and on animal health. In individual cases, "cross-talk" may protect the plant against other forms of stressors, including diseases. Nevertheless, each case needs to be evaluated individually and given the complexity of the traits and the interaction with plant inherent metabolic pathways, advanced testing may be required;

- Some research strategies involve stress response metabolites that are known to have anti-nutritional and allergenic properties. If these would be further developed, the potential risk to consumers will need to be thoroughly considered. Another possible scenario includes that by protecting a plant against a stressor, other stress responses or metabolic pathways native to plant produce undesired compounds. Under normal conditions these would not become available as the plant may not survive the stress factor. Risk assessment approaches that are in place are expected to adequately address such concerns.

- Management practice that would extend the growing area of the crop potentially could have an important impact on the local ecosystems.

Although some of these indications require new ways to collect and present safety information, all issues identified in this study can be addressed based on the structure of the ERA according to Annex II.D.2. Eventually the ERA will be depending on the nature of the modified crop, the

characteristics of the introduced trait, the potential receiving environment and the likely interactions. Fundamental research can contribute to improving the framework for the ERA by:

- Further documenting the mechanisms of and relationship between the different response pathways. This will support delineating the possible changes that occur when introducing a particular function and help product characterization. Nevertheless it has been argued that detailed and robust phenotypic comparisons can provide adequate information for an ERA.
- Exploring which (combination of) factors influence “weediness” of domesticated species. While further general insights are generated, observing the effect of an individual trait may be sufficient within the context of an ERA.

Finally, it should also be noted that –while of agronomic importance- many of the traits also may have a positive environmental effect. Growing plants which are less dependent on water availability, using nutrients more efficiently, preventing yield losses, producing on sub-optimal soils, are just a few of the goals that are heralded as possibly contributing to food security.

6 References - Literature

Abe H., Urao T., Ito T., Seki M., Shinozaki K., Yamaguchi-Shinozaki K. (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) Function as Transcriptional Activators in Abscisic Acid Signaling. The Plant Cell 15: 63-78.

Ambrose B.A., Lerner D.R., Ciceri P., Padilla C.M., Yanofsky M.F. and Schmidt R.J. (2000) Molecular and Genetic Analyses of the Silky1 Gene Reveal Conservation in Floral Organ Specification between Eudicots and Monocots. Molecular Cell 5: 569–579.

Ammann K., Jacot Y. and Rufener Al Mayzad P. (2000) Weediness in the light of new transgenic crops and their potential hybrids, Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Sonderheft XVII; http://www.botanischergarten.ch/Weeds/weeds1.pdf (last accessed 20 December 2009)

Ampong-Nyarko K. and de Datta S.K. (1993) Effects of nitrogen application on growth, nitrogen use efficiency and rice-weed interaction. Weed Res. 33: 269– 276.

Amzallag G.N. (1999) Plant evolution an adaptive theory. In: Plant Responses to Environmental Stresses. Ed. H.R. Lerner, M. Dekker, New York, 171–246.

APHIS-USDA, December 17, 2009, Revised Draft Environmental Assessment: Permit applications 08-011-116rm and 08-014-101rm received from ArborGen LLC; Field testing of genetically engineered *Eucalyptus grandis* X *Eucalyptus urophylla.* http://www.aphis.usda.gov/brs/aphisdocs/08_014101rm_ea2.pdf

APHIS-USDA, June 27, 2007, Finding of No Significant Impact and Decision Notice; Issuance of a permit to continue research on genetically engineered *Eucalyptus* hybrids. http://www.aphis.usda.gov/brs/aphisdocs/06_325111r_ea.pdf

APHIS-USDA, May 8, 2009, Draft Environmental Assessment: Permit applications 08-011-116rm and 08-014-101rm received from ArborGen LLC; Field testing of genetically engineered *Eucalyptus grandis* X *Eucalyptus urophylla.* http://www.aphis.usda.gov/brs/aphisdocs/08_014101rm_ea.pdf

Apse M.P. and Blumwald E. (2007) Na^+ transport in plants. FEBS Letters 581: 2247–2254.

Ashikari M., Sakakibara H., Lin S., Yamamoto T., Takashi T., Nishimura A., Angeles E.R., Qian Q., Kitano H. and Matsuoka M. (2005) Cytokinin oxidase regulates rice grain production. Science 309: 741-745.

Babu R.C., Zhang J., Blum A., Ho D.T.H., Wu R. and Nguyen H.T. (2004) HVA1, a LEA gene from barley confers dehydration tolerance in transgenic rice (*Oryza sativa* L.) via cell membrane protection. Plant Sci 166: 855–862.

Bae G. and Choi G. (2008) Decoding of Light Signals by Plant Phytochromes and Their Interacting Proteins. Annu. Rev. Plant Biol. 59: 281-311.

Baker H.G. (1965) Characteristics and modes of origin of weeds. In H.G. Baker and G.L. Stebbins (Eds.), The Genetics of Colonizing Species (pp. 147–172). New York: Academic Press.

Baker H.G. (1974) The evolution of weeds. Annu. Rev. Ecol. Syst. 5: 1–24.

Baker H.G. (1991) The continuing evolution of weeds. Econ. Bot. 45: 445–449.

Bartels D. and Sunkar R. (2005) Drought and Salt Tolerance in Plants, Critical Reviews in Plant Sciences 24(1): 23-58.

Baulcombe D. (2004) Review article: RNA silencing in plants Nature 431, 356-363

Beckie H.J. and Hall L.M. (2008) Simple to complex: modelling crop pollen-mediated gene flow. Plant Sci. 175: 615–628.

Beemster G.T.S., Mironov V. and Inze D. (2005) Tuning the cell-cycle engine for improved plant performance. Current Opinion in Biotechnology 16: 142–146.

Ben Saad R., Zouari N., Ben Ramdhan W., Azaza J., Meynard D., Guiderdoni E. and Hassairi A. (2010) Improved drought and salt stress tolerance in transgenic tobacco over-expressing a novel A20/AN1 zinc-finger "AlSAP" gene isolated from the halophyte grass *Aeluropus littoralis*. Plant Mol. Biol. 72: 171-190.

Bergelson J. (1994) Changes in fecundity do not predict invasiveness: a model study of transgenic plants. Ecology 75: 249–252.

Bergelson J. and Purrington C.B. (1996) Surveying patterns in the cost of resistance in plants. Am. Nat. 148: 536–558.

Biemelt S., Tschiersch H., and Sonnewald U. (2004) Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. Plant Physiology 135: 254–265.

Blázquez M.A., Ferrandiz C., Madueno F., Parcy F. (2006) How floral meristems are built. Plant Mol. Biol. 60: 855–870.

Blumwald E., Grover A., and Good A.G. (2004). Breeding for abiotic stress resistance: challenges and opportunities. In "New directions for a diverse plant". Proceedings of the 4th International Crop Science Congress, 26 Sep - 1 Oct 2004, Brisbane, Australia": http://www.cropscience.org.au/icsc2004/pdf/1953_blumwalde.pdf.

Bradford K.J., van Deynze A., Gutterson N., Parrot W. and Strauss, S.H.(2005) Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. Nature Biotechnology 23(4): 439-444.

Braidek J.T., Fedec P. and Jones D. (1984) Field survey of halophytic plants of disturbed sites on the Canadian Prairies. Can. J. Plant Sci. 64: 745–752.

Brar D.S. and S.M. Jain (1998) Somaclonal variation, mechanisms and applications in crop improvement. In: Somaclonal variation and induced mutations in crop improvement. Kluwer, Dordrecht,Eds. S.M. Jain, D.S.Brar, B.S. Alhoowalia.

Brini F., Hanin M., Lumbreras V., Amara I., Khoudi H., Hassairi A., Pagès M. and Masmoudi K. (2007) Overexpression of wheat dehydrin DHN-5 enhances tolerance to salt and osmotic stress in *Arabidopsis thaliana*. Plant Cell Rep 26: 2017–2026.

Brugière N., Humbert S., Rizzo N., Bohn J. and Habben J.E. (2008) A member of the maize isopentenyl transferase gene family, Zea mays isopentenyl transferase 2 (*ZmIPT2*), encodes a cytokinin biosynthetic enzyme expressed during kernel development. Plant Mol Biol 67: 215–229.

Buck M.J. and Atchley W.R. (2003) Phylogenetic analysis of plant basic helix-loop-helix proteins. J. Mol. Evol. 56: 742-750.

Bülow L., Holmberg N., Lilius G. and Bailey J.E. (1999) The metabolic effects of native and transgenic hemoglobins on plants. Trends in Biotechnology 17: 21-24.

Burke J.M. (2004) When good plants go bad. Evolution, 58, 1637–1638.

Burke J.M. and Rieseberg L.H. (2003) Fitness effects of transgenic disease resistance in sunflowers. Science 300: 1250.

Busov V.B., Meilan R., Pearce D.W., Ma C., Rood S.B. and Strauss S.H. (2003) Activation Tagging of a dominant gibberellin catabolism gene (GA 2-oxidase) from poplar that regulates tree stature. Plant Physiology 132: 1283–1291.

Calderini O., Bovone T., Scotti C., Pupilli F., Piano E. and Arcioni S. (2006) Delay of leaf senescence in *Medicago sativa* transformed with the *ipt* gene controlled by the senescence-specific promoter SAG12. Plant Cell Rep. 26(5): 611-615.

Campbell L.G. and Snow, A.A. (2006) Competition alters life history and increases the relative fecundity of crop-wild radish hybrids (Raphanus spp.). New Phytol. 173: 648–660.

Capell T., Bassie L. and Christou P. (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. PNAS 101(26): 9909-9914.

Carlson H.L. and Hill J.E. (1985) Wild oat (*Avena fatua*) competition with spring wheat: effects of nitrogen fertilization. Weed Sci. 34: 29–33.

Cassells A.C. and B. M. Doyle (2003) Genetic engineering and mutation breeding for tolerance to abiotic and biotic stresses: science, technology and safety. Bulg. J. Plant Physiol., Special Issue 2003, 52–82.

Castiglioni P., Warner D., Bensen R.J., Anstrom D.C., Harrison J., Stoecker M., Abad M., Kumar G., Salvador S., D'Ordine R., Navarro S., Back S., Fernandes M., Targolli J., Dasgupta S., Bonin C., Luethy M.H. and Heard J.E. (2008) Bacterial RNA Chaperones Confer Abiotic Stress Tolerance in Plants and Improved Grain Yield in Maize under Water-Limited Conditions. Plant Physiology 147: 446–455.

Century K., Reuber T.L. and Ratcliffe O.J. (2008) Regulating the Regulators: The Future Prospects for Transcription-Factor-Based Agricultural Biotechnology Products. Plant Physiol. 147: 20–29.

CGB (2003) Avis sur B/FR/03.02.04 "Validation fonctionnelle d'un gène impliqué dans les mécanismes d'assimilation de l'azote et de remplissage du grain"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2003/AV030204.pdf

CGB (2005a) Avis sur B/FR/05.01.01 "Validation fonctionnelle d'un gène impliqué dans les mécanismes d'assimilation de l'azote et de remplissage du grain";

http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2005/AvisBFR050101.pdf

CGB (2005b) Avis sur B/FR/05.02.03 "Amélioration des performances photosynthétiques de la plante en conditions de stress hydrique"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2005/avisBFR050203.pdf

CGB (2006a) Avis sur B/FR/06.01.13 "Essais au champs de maïs génétiquement modifiés pour l'amélioration des performances photosynthétiques de la plante en conditions de stress hydrique"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2006/BFR060113-Biogemma.pdf

CGB (2006b) Avis sur B/FR/06.02.03 "Essais au champs de maïs génétiquement modifiés pour le caractère de précocité de floraison"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2006/BFR060203.pdf

CGB (2006c) Avis sur B/FR/06.12.05 "Essais au champs de maïs génétiquement modifiés pour le caractère de précocité de floraison"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2006/seance20061212-13/BFR061205.pdf

CGB (2006d) Avis sur B/FR/06.12.06 "Essais au champs de maïs génétiquement modifiés pour l'amélioration des performances photosynthétiques de la plante en conditions de stress hydrique"; http://www.ogm.gouv.fr/experimentations/evaluation_scientifique/cgb/avis_dossiers_2006/seance20061212-13/BFR061206.pdf

Chapman M.A. and Burke J.M. (2006) Letting the gene out of the bottle: the population genetics of genetically modified crops. New Phytol. 170: 429–443.

Chaves M.M. and Oliveira M.M. (2004) Mechanisms underlying plant resistance to water deficits: prospects for water-saving agriculture. J Exp. Bot. 55: 2365-2384.

Chen J.-Q., Meng X.-P., Zhang Y., Xia M.and Wang X.-P. (2008) Over-expression of OsDREB genes lead to enhanced drought tolerance in rice. Biotechnology Letters 30(12): 2191-2198.

Chen T.H.H., and N. Murata (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes Current Opinion in Plant Biology 5(3): 250-257

Chèvre A.-M., Ammitzbøll H., Breckling B., Dietz-Pfeilstetter A., Eber F., Fargue A., Gomez-Campo C., Jenczewski E., Jørgensen R.B., Lavigne C., Meier M.S., den Nijs H.C.M., Pascher K., Séguin-Swartz G., Sweet J., Stewart Jr. C.N. and Warwick S. (2004) A review on interspecific gene flow from oilseed rape to wild relatives. In H.C.M. den Nijs, D. Bartsch, and J. Sweet (Eds.), Introgression from Genetically Modified Plants into Wild Relatives (pp. 235–251). Wallingford, Oxfordshire, UK: CABI Publishing.

Chiang G.C.K., Barua D., Kramer E.M., Amasino R.M. and Donohue K. (2009) Major flowering time gene, FLOWERING LOCUS C, regulates seed germination in *Arabidopsis thaliana*. PNAS 106 (28): 11661-11666.

CNB, 2008, Acta de la sexagésimo tercera reunión de la comisión nacional de bioseguridad; http://www.mma.es/secciones/calidad_contaminacion/omg/cnb/pdf/cnb_acta_63.pdf

Coen E.S., and Meyerowitz E.M. (1991) The war of the whorls: genetic interactions controlling flower development. Nature 353: 31–37.

Colautti R.I. and MacIsaac H.J. (2004) A neutral terminology to define 'invasive' species. Diversity & Distributions 10(2): 135-141.

Coles J.P., Phillips A.L., Croker S.J., Garcia-Lepe R., Lewis M.J. and Hedden P. (1999) Modification of gibberellin production and plant development in *Arabidopsis* by sense and antisense expression of gibberellin 20-oxidase genes. Plant J. 17: 547–556.

Connolly E.L., Campbell N.H., Grotz N., Prichard C.L, and Guerinot M.L. (2003) Overexpression of the FRO2 Ferric Chelate Reductase Confers Tolerance to Growth on Low Iron and Uncovers Posttranscriptional Control. Plant Physiology 133: 1102–1110.

Coruzzi G. and Bush D.R. (2001) Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiol 125: 61–64.

Crawley M.J., Hails R.S., Rees M., Kohn D. and Buxton J. (1993) Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620–623.

Csukasi F., Merchante C. and Valpuesta V. (2009) Modification of plant hormone levels and signaling as a tool in plant biotechnology. Biotechnology Journal 4(9): 1293-1304.

Cummings C.L. and Alexander H.M. (2002) Population ecology of wild sunflowers: effects of seed density and post-dispersal vertebrate seed predation on numbers of plants per patch and seed production. Oecologia 130: 272–280.

Dan I., Watanabe N.M. and Kusumi A. (2001) The Ste20 group kinases as regulators of MAP kinase cascades. Trends Cell Biol. 11: 220-230.

Darley C.P., Van Wuytswinkel O.C.M., Van der Woude K., Mager W.H. and A.H. De Boer (2000) *Arabidopsis thaliana* and *Saccharomyces cerevisiae* NHX1 genes encode amiloride sensitive electroneutral Na^+/H^+ exchangers. Biochem. J. 351: 241-249.

de Jong T.J. and P.G.L. Klinkhamer (2005) Evolutionary ecology of plant reproductive strategies. Cambridge University Press. 302 pp. ISBN 100-521-82142-8

Delhaize E., Ryan P.R., Hebb D.M., Yamamoto Y., Sasaki T. and Matsumoto H. (2004) Engineering high-level aluminum tolerance in barley with *ALMT1* gene. PNAS 101: 15249–15254.

Dezar C.A., Gago G.M., Gonzalez D.H. and Chan R.L. (2005) Hahb-4, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. Transgenic Res. 14(4): 429-40.

DiTommaso A. (2004) Germination behavior of common ragweed (*Ambrosia artemisiifolia*) populations across a range of salinities.Weed Sci. 52: 1002–1009.

Dubcovsky J. (2004), Marker-Assisted Selection in Public Breeding Programs: The Wheat Experience Crop Sci. 44: 1895-1898.

Dudits D., Cserhári M., Miskoleze P. and Horváth G.V. (2007) The growing family of plant cyclin-dependent kinases with multiple functions in cellular and developmental regulation. In 'Cell cycle control and plant development. Annual Plant Reviews, Volume 32' Ed. D. Inzé. Oxford: Wiley-Blackwell. 364 pp. ISBN 978-1-4051-5043-9.

Dunwell J.M. (2000) Transgenic approaches to crop improvement. J. Exp. Bot. 51: 487-496.

EFSA (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100.

EFSA (2010) Scientific Opinion on the assessment of potential impacts of genetically modified plants on non-target organisms. Public consultation document available at www.efsa.europa.eu. Last accessed 10 March 2010.

Ellstrand N.C., Prentice H.C. and Hancock J.F. (1999) Gene flow and introgression from domesticated plants into their wild relatives. Annu. Rev. Ecol. Syst. 30: 539–563.

Ellstrand N.C. (2001) When transgenes wander, should we worry? Plant Physiol., 125, 1543–1545.

Ellstrand N.C. and Hoffman C.A. (1990) Hybridization as an avenue for escape of engineered genes. Bioscience 40: 438–442.

Etherington E., Gandhdhi H., Busov V., Meilalan R., Ma C., Kosolala K., and S.H. Strauss (2007) Dwarfism Genes for Modifying the Stature of Woody Plants: A Case Study in Poplar. Landscape Plant News, Spring, 3-6.

Eveno E., Collada C. and Guevara M.A. (2008) Contrasting patterns of selection at *Pinus pinaster* Ait. drought stress candidate genes as revealed by genetic differentiation analyses. Mol. Biol. Evol. 25: 417–437.

FAO (2001) Glossary of Biotechnology for Food and Agriculture; http://www.fao.org/biotech/index_glossary.asp

FAO (2003) Applications of Molecular Biology and Genomics to Genetic Enhancement of Crop Tolerance to Abiotic Stress: A Discussion Document. interim SCIENCE COUNCIL SECRETARIAT FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. September 2003. ftp://ftp.fao.org/docrep/nonfao/tac/Y5198E/Y5198E00.pdf (last accessed 20 December 2009).

Fei H., Chaillou S., Hirel B., Polowick P., Mahon J.D. and Vessey J.K. (2006) Effects of the over-expression of a soybean cytosolic glutamine synthetase gene (GS15) linked to organ-specific promoters on growth and nitrogen accumulation of pea plants supplied with ammonium. Plant Physiol. Biochem. 44: 543-550.

Flowers T.J. (2004) Improving crop salt tolerance. Journal of Experimental Botany 55(396): 307-319.

Forcella F. (1985) Final distribution is related to rate of spread in alien weeds. Weed Res. 25: 181–191.

Franks S.J., Pratt P.D., Dray F.A. and Simms E.L. (2008) Selection on herbivory resistance and growth rate in an invasive plant. Am. Nat. 171: 678–691.

Friesen L.F., Beckie H.J., Warwick S.I. and R.C. Van Acker (2009) The biology of Canadian weeds. *Kochia scoparia* (L.) Schrad. Can. J. Plant Sci. 89: 141–167.

Fuentes S.I., Allen D.J., Ortiz-Lopez A. and Hernández G. (2001) Over-expression of cytosolic glutamine synthetase increases photosynthesis and growth at low nitrogen concentrations. J. Exp. Botany 52: 1071-1081.

Funk J.L. and Vitousek P.M. (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446: 1079–1081.

Gallardo F., Fu J., Cantón F.R., García-Gutiérrez A., Cánovas F.M., Kirby E.G. (1999) Expression of a conifer glutamine synthetase gene in transgenic poplar. Planta 210: 19–26.

Gallavotti A., Yang Y., Schmidt R.J. and Jackson D. (2008) The Relationship between Auxin Transport and Maize Branching. Plant Physiol. 147(4): 1913–1923.

Gan S. and Amasino, R.M. (1995) Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270: 1986–1988.

Gaxiola R.A., Li J., Undurraga S., Dang L.M., Allen G.J., Alper S.L. and Fink G.R. (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H1-pump. PNAS 98520: 11444–11449.

Ge L.-F., Chao D.-Y., Shi M., Zhu M.-Z., Gao J.-P., Lin H.-X. (2008) Over-expression of the trehalose-6-phosphate phosphatase gene OsTPP1 confers stress tolerance in rice and results in the activation of stress responsive genes. Plant 228: 191-201.

Geitmann A., and Ortega J.K.E. (2009) Mechanics and modeling of plant cell growth. Trends in Plant Sci 14(9): 467-478.

Goddijn O.J. and Van Dun K., (1999) Trehalose metabolism in plants. Trends Plant Sci. 4(8): 315-319.

Goddijn O.J.M., Verwoerd T.C., Voogd E., Krutwagen R.W.H.H., de Graaf P.T.H.M., Poels J., Van Dun K., Ponstein A.S., Damm B. and Pen J. (1997) Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol. 113: 181–190.

Good A.G., Johnson S.J., De Pauw M., Carroll R.T., Savidov N., Vidmar J., Lu Z., Taylor G. and Stroeher V. (2007) Engineering nitrogen use efficiency with alanine aminotransferase. Canadian Journal of Botany 85: 252–262.

Good A.G., Shrawat A.K. and Meunch D.G. (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci. 9(12): 597-605.

Grant V. (1981). Plant Speciation (2nd ed.). New York: Columbia University Press.

Gray A.J. (2004) Ecology and government policies: the GM crop debate. J. Appl. Ecol. 41: 1−10.

Halfhill M.D., Sutherland, J. P.,Moon,H. S., Poppy G.M., Warwick S.I., Weissinger A. K., Rufty T.W., Raymer P.L. and C.N. Stewart (2005) Growth, productivity, and competitiveness of introgressed weedy Brassica rapa hybrids selected for the presence of Bt cry1AC and gfp transgenes. Mol. Ecol. 14: 3177–3189.

Harding S.A. and Smigocki A.C. (1994) Cytokinins modulate stress response genes in isopentenyl transferase-transformed Nicotiana plumbaginifolia plants. Physiologia Plantarum 90: 327-333.

Haslberger A.G. (2003) Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21: 739-741.

Häusler R.E., Hirsch H.-J., Kreuzaler F. and Peterhänsel P. (2002) Over expression of C_4-cycle enzymes in transgenic C_3 plants: a Biotechnological approach to improve C_3-photosynthesis.

Haygood R., Ives A.R. and Andow D.A. (2004) Population genetics of transgene containment. Ecol. Lett. 7: 213–220.

He C. (2005) Analysis of AtNHX1-expressing transgenic cotton under high salt conditions and in the field. Dissertation in Biology, Texas Tech University, 106p.

Hill R.A. (2005) Conceptualizing risk assessment methodology for genetically modified organisms. Environ Biosafety Res 4(2): 67–70.

Hirel B., Le Gouis J., Ney B. and Gallais A. (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58: 2369-2387.

Hoekenga O.A. (2008) Using Metabolomics To Estimate Unintended Effects in Transgenic Crop Plants: Problems, Promises, and Opportunities. Journal of Biomolecular Techniques 19: 159–166.

Holmberg N., Lilius G., Bailey J.E. and Bülow L. (1997) Transgenic tobacco expressing *Vitreoscilla* hemoglobin exhibits enhanced growth and altered metabolite production. Nature Biotechnology 15: 244-247.

Hong Z., Ueguchi-Tanaka M., Fujioka S., Takatsuto S., Yoshida S., Hasegawa Y., Ashikari M., Kitano H. and Matsuoka M. (2005) The Rice brassinosteroid-deficient dwarf2 Mutant, Defective in the

Rice Homolog of *Arabidopsis* DIMINUTO/DWARF1, Is Rescued by the Endogenously Accumulated Alternative Bioactive Brassinosteroid, Dolichosterone. Plant Cell 17: 2243-2254.

Husaineid S.S.H. (2007) Genetic modification of shade-avoidance: over-expression of homologous phytochrome genes in tomato. PhD thesis, Wageningen University, Wageningen, The Netherlands.

Igamberdiev A.U., Baron K., Manac'h-Little N., Stoimenova M. and Hill R.D. (2005) The Haemoglobin/Nitric Oxide Cycle: Involvement in Flooding Stress and Effects on Hormone Signalling. Annals of Botany 96: 557-564.

Inzé D. and De Veylder L. (2006) Cell Cycle Regulation in Plant Development. Annu. Rev. Genet. 40: 77-105.

Jackson M.W., Stinchcomb, J.R., Korves T.M. and J. Schmitt. (2004) Costs and benefits of cold tolerance in transgenic *Arabidopsis thaliana*. Mol. Ecol. 13: 3609–3615.

James V.A., Neibaur I., Altpeter F. (2008) Stress inducible expression of the DREB1A transcription factor from xeric, *Hordeum spontaneum* L. in turf and forage grass (*Paspalum notatum* Flugge) enhances abiotic stress tolerance. Transgenic Res 17: 93–104.

Jing Z.P., Gallardo F., Belén Pascual M., Sampalo R., Romero J., Torres de Navarra A. and Cánovas F.M., 2004, Improved growth in a field trial of transgenic hybrid poplar over-expressing glutamine synthetase. New Phytologist 164: 137–145.

Jung C. and Müller A.E. (2009) Flowering time control and applications in plant breeding. Trends in Plant Sci 14(10): 563-573.

Kahl G. (2001) The dictionary of gene technology: genomics, transcriptomics, proteomics. Wiley-VCH Weinheim, Germany. pp 1-941.

Kalifa Y., Perlson E., Gilad A., Konrad Z., Scolnik P.A. and Bar-Zvi D. (2004) Over-expression of the water and salt stress-regulated *Asr1* gene confers an increased salt tolerance. Plant, Cell and Environment 27: 1459–1468.

Kane N.C., Rieseberg L.H. (2007) Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. Genetics 175: 1823–1834.

Karaba A., Dixit S., Greco R., Aharoni A., Trijatmiko K.R., Marsch-Martinez N., Krishnan A., Nataraja K.N., Udayakumar M. and Pereira A. (2007) Improvement of water use efficiency in rice by expression of *HARDY*, an *Arabidopsis* drought and salt tolerance gene. PNAS 104(39): 15270–15275.

Kasukabe Y., He L., Nada K., Misawa S., Ihara I., Tachibana S. (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 45: 712-722.

Katiyar-Agarwal S., Agarwal M. and Grover A. (2003) Heat-tolerant basmati rice engineered by over-expression of *hsp101*. Plant Mol Biol. 51(5): 677-686.

Kebeish R., Niessen M., Thiruveedhi K., Bari R., Hirsch H.J., Rosenkranz R., Stäbler N., Schönfeld B., Kreuzaler F. and Peterhänsel Ch. (2007) Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nat. Biotech. 25: 593–599.

Keeler K.H. (1989) Can genetically engineered crops become weeds? Bio/Technology 7: 1134-1139.

Klempnauer K.-H., Gonda T.J. and Bishop J.M. (1982) Nucleotide sequence of the retroviral leukemia gene v-myb and its cellular progenitor c-myb: The architecture of a transduced oncogene. Cell 31(2): 453-463.

Knight H. and Knight M.R. (2001) Abiotic stress signalling pathways: specificity and cross-talk. TRENDS in Plant Science 6(6): 262 – 267.

Knispel A.L., McLachlan S.M., Van Acker R.C. and Friesen L.F. (2008) Gene flow and multiple herbicide resistance in escaped canola populations. Weed Sci. 56: 72–80.

Korves T.M. and Bergelson J. (2004) A novel cost of R gene resistance in the presence of the disease. Am.Nat. 163: 489–504.

Krizek B.A. (2009) Making bigger plants: key regulators of final organ size. Curr. Opin. Plant Biol. 12: 17-22.

Kurland C.G., Canback B. and Berg O.G. (2003) Horizontal gene transfer: a critical view. PNAS 100: 9658-9662.

Lai Z., Kane N.C., Zou, Y. and Rieseberg L.H. (2008) Natural variation in gene expression between wild and weedy populations of Helianthus annuus. Genetics 179: 1881–1890.

Landoni M., Cassani E. and Pilu R. (2007) *Arabidopsis thaliana* plants over-expressing Ramosa1 maize gene show an increase in organ size due to cell expansion. Sexual Plant Reproduction 20 (4): 191-198.

Lawis J.M., Mackintosh C.A., Shin S., Gilding E., Kravchenko S., Baldridge G., Zeyen R. and Muehlbauer G.J. (2008) Over-expression of the maize *Teosinte Branched1* gene in wheat suppresses tiller development. Plant Cell Rep 27: 1217–1225.

Lea P.J. and Azevedo R.A. (2006) Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. Annals of Applied Biology 149(3): 243–247.

Lea P.J. and Azevedo R.A. (2007) Nitrogen use efficiency. 2. Amino acid metabolism. Annals of Applied Biology 151(3): 269–275.

Lee B.-H., Johnston R., Yang Y., Gallavotti A., Kojima M., Travencolo B.A.N., da F. Costa L., Sakakibara H. and Jackson D. (2009) Studies of abphyl1 phyllotaxy mutants of maize indicate complex interactions between auxin and cytokinin signaling in the shoot apical meristem. Plant Physiol. 150: 205-216.

Lefebvre S., Lawson T., Zakhleniuk O.V., Lloyd J.C. and Raines C.A. (2005) Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. Plant Physiol. 138: 451-460.

Lehesranta S.J., Davies H.V., Shepherd LV.T., Nunan N., McNicol J.W., Auriola S., Koistinen K.M., Suomalainen S., Kokko H.I. and Karenlampi S.O. (2005). Comparison of Tuber Proteomes of Potato Varieties, Landraces, and Genetically Modified Lines. Plant Physiology 138: 1690-1699.

Lerner H.R. (1999) Introduction to the response of plants to environmental stresses. In:Plant Responses to Environmental Stresses. Ed. H.R. Lerner. Marcel Dekker, New York, 1–26.

Li Q., Robson P.R.H., Bettany A.J.E., Donnison I.S., Thomas H. and Scott I.M. (2004) Modification of senescence in ryegrass transformed with IPT under the control of a monocot senescence-enhanced promoter. Plant Cell Rep. 22(11): 816-821.

Li Q.L., Gao X.R., Yu X.H., Wang X.Z., An L.J. (2003) Molecular cloning and characterization of betaine aldehyde dehydrogenase gene from *Suaeda liaotungensis* and its use in improved tolerance to salinity in transgenic tobacco. Biotechnol Lett 25: 1431-1436.

Long S., Zhu X.-G., Naidu S.L. and Ort D.R. (2006) Can improvement in photosynthesis increase crop yields? Plant, Cell and Environment 29: 315–330.

Losos J.B. and De Queiroz K. (1997) Evolutionary consequences of ecological release in Caribbean Anolis lizards. Biological Journal of the Linnean Society. 61: 459-483.

Lu B.-R. (2008) Transgene Escape from GM Crops and Potential Biosafety Consequences: An Environmental Perspective ICGEB Collection of Biosafety Reviews Vol. 4: 66-141.

Lu B.-R. and Snow A.A. (2005) Gene Flow from Genetically Modified Rice and Its Environmental Consequences. BioScience 55(8): 669–678.

Mallory-Smith C. and Zapiola M. (2008) Gene flow from glyphosate-resistant crops. Pest Manag. Sci. 64: 428–440.

Martin A., Lee J., Kichey T., Gerentes D., Zivy M., Tatout Ch., Dubois F., Balliau T., Valot B., Davanture M., Tercé-Laforgue T., Quilleré I., Coque M., Gallais A., Gonzalez-Moro M.-B., Bethencourt L., Habash D., Lea P.J., Charcosset A., Perez P., Murigneux A., Sakakibara H. Edwards K.J. and Hirel B. (2006) Two cytosolic glutamine synthetase isoforms of maize are specifically involved in the control of grain production. The Plant Cell 18: 3252–3274.

Mason P., Braun L., Warwick S.I., Zhu B. and Stewart Jr. C.N. (2003) Transgenic Bt-producing *Brassica napus*: *Plutella xylostella* selection pressure and fitness of weedy relatives. Environ. Biosafety Res. 2: 263–276.

Maurel C. (2007) Plant aquaporins: Novel functions and regulation properties. FEBS Letters 581: 2227–2236.

McSteen P. and Leyser O. (2005) Shoot branching. Annu Rev Plant Biol 56: 353-374.

Mehta R.A., Cassol T., Li N., Ali N., Handa A.K. and Mattoo A.K. (2002) Engineered polyamine accumulation in tomato enhances phytonutrient content, juice quality, and vine life. Nat Biotechnol 20: 613-618.

Mercer K.L., Andow D.A., Wyse D.L. and Shaw R.G. (2007) Stress and domestication traits increase the relative fitness of crop-wild hybrids in sunflower. Ecology Letters 10(5): 383-393.

Michaels S.D. (2009) Flowering time regulation produces much fruit. Current Opinion in Plant Biology 12: 75–80.

Mimura M., Lelmen K.E., Shimazaki T., Kikuchi A. and Watanabe K.N. (2008) Impact of Environmental Stress-Tolerant Transgenic Potato on Genotypic Diversity of Microbial Communities and Soil Enzyme Activities under Stress Conditions. Microbes and Environments 23(3): 221-228.

Miyagawa Y., Tamoi M., Shigeoka S. (2001) Over-expression of a cyanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. Nat. Biotechnol. 19: 965–969.

MMA, 2007, Evaluación de riesgo de la liberación en campo de citrange carrizo modificado geneticamente (B/ES/06/43); http://www.mma.es/secciones/calidad_contaminacion/omg/notificaciones_autorizaciones/pdf/ERMA_B_ES_06_43.pdf

MMA, 2008a, Evaluación de riesgo de la liberación en campo de citrange carrizo modificado geneticamente (B/ES/08/03); http://www.mma.es/secciones/calidad_contaminacion/omg/notificaciones_autorizaciones/pdf/ERMA_B_ES_08_03.pdf

MMA, 2008b, Evaluación de riesgo de la liberación en campo de naranjo modificado geneticamente (B/ES/08/05); http://www.mma.es/secciones/calidad_contaminacion/omg/notificaciones_autorizaciones/pdf/ERMA_B_ES_08_05.pdf

MMA, 2009, Evaluación de riesgo de la liberación en campo de plantas de patata modificadas geneticamente (B/ES/09/57); http://www.mma.es/secciones/calidad_contaminacion/omg/notificaciones_autorizaciones/pdf/ERMA_B_ES_09_57.pdf

Mohanty A., Kathuria H., Ferjani A., Sakamoto A., Mohanty P., Murata N., Tyagi A.K. (2002) Transgenics of an elite *indica* rice variety Pusa Basmati 1 harbouring the *codA* gene are highly tolerant to salt stress. Theor Appl Genet. 106: 51-57.

Møller I.S., Gilliham M., Jha D., Mayo G.M., Roy S.J., Coates J.C., Haseloff J., and Tester M. (2009). Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na^+ transport in *Arabidopsis*. The Plant Cell 21: 2163-2178.

Moose S. (2009) Doubling a Gene in Corn Results in Giant Biomass (published March 2009, last consulted 15 December 2009); http://www.aces.uiuc.edu/news/stories/news4683.html

Motavalli P.P., Kremer R.J., Fang M. and Means N.E. (2004) Impact of Genetically Modified Crops and Their Management on Soil Microbially Mediated Plant Nutrient Transformations J. Environ. Qual. 33: 816–824.

Müller B. and Sheen J. (2007) Advances in Cytokinin Signaling. Science 318: 68-69.

Munns R. and Tester M. (2008) Mechanisms of Salinity Tolerance. Annu. Rev. Plant Biol. 59: 651–681.

Mutasa-Göttgens E. and Hedden P. (2009) Gibberellin as a factor in floral regulatory networks. J Exp Bot. 60(7): 1979-1989.

Nanjo T., Kobayashia M., Yoshibab Y., Kakubaric Y., Yamaguchi-Shinozaki K. and Shinozaki K. (1999) Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. FEBS Lett 461: 205–210.

Nelson D.E., Repetti P.P., Adams T.R., Creelman R.A., Wu J., Warner D.C., Anstrom D.C., Bensen R.J., Castiglioni P.P., Donnarummo M.G., Hinchey B.S., Kumimoto R.W., Maszle D.R., Canales R.D., Krolikowski K.A., Dotson S.B., Gutterson N., Ratcliffe O.J. and Heard J.E. (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. PNAS 104: 16450–16455.

Nickson T.E. (2008) Planning Environmental Risk Assessment for Genetically Modified Crops: Problem Formulation for Stress-Tolerant Crops. Plant Physiol. 147: 494-502.

Nilsson L., Muller R. and Nielsen T.H. (2007) Increased expression of the MYB-related transcription factor, PHR1, leads to enhanced phosphate uptake in *Arabidopsis thaliana*. Plant, Cell and Environment 30: 1499-1512.

NRC (2002) Environmental effects of transgenic plants: the scope and adequacy of regulation, National Research Council, National Academy Press, Washington DC, ISBN 0-309-08263-3.

OGTR (2005) DIR 053/2004 - Full Risk Assessment and Risk Management Plan for Field trial of genetically modified salt tolerant wheat on saline land; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir053-2004

OGTR (2006a) DIR 064/2006 - Full Risk Assessment and Risk Management Plan (RARMP) for the Limited and Controlled Release of Water-Efficient GM Cotton;

http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir064-2006
OGTR (2006b) DIR 067/2006 - Full Risk Assessment and Risk Managment Plan (RARMP) for the Limited and Controlled Release of Waterlogging Tolerant Genetically Modified (GM) Cotton; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir067-2006
OGTR (2007a) DIR 070/2006 - Full Risk Assessment and Risk Management Plan (RARMP) for Limited and Controlled Release of Genetically Modified (GM) Sugarcane with altered plant architecture, enhanced water or improved nitrogen use efficiency; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir070-2006
OGTR (2007b) DIR 071/2006 - Full Risk Assessment and Risk Management Plan (RARMP) for Limited and Controlled Release of GM drought tolerant wheat; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir071-2006
OGTR (2008a) DIR 077/2007 - Full Risk Assessment and Risk Management Plan (RARMP) for Limited and Controlled Release of Wheat and Barley Genetically Modified for Enhanced Tolerance to Abiotic Stresses or Increased beta glucan; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir077-2007
OGTR (2008b) DIR 080/2007 – Full Assessment and Risk Management Plan (RARMP) for Limited and Controlled Release of Wheat Genetically Modified for Drought Tolerance; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir080-2007
OGTR (2008c) DIR 081/2007 - Full Risk Assessment and Risk Management Plan (RARMP) for Limited and Controlled Release of Cotton Genetically Modified for enhanced water use efficiency; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir081-2007
OGTR (2008d) DIR 083/2007 - Full Risk Assessment and Risk Management Plan (RARMP) for limited and controlled release of cotton genetically modified for enhanced waterlogging tolerance; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir083-2007
OGTR (2008e) DIR 084/2008 – Full Risk Assessment and Risk Management Plan (RARMP) for limited and controlled release of torenia genetically modified for enhanced phosphate uptake; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir084-2008
OGTR (2009a) DIR 094/2009 - Full Risk Assessment and Risk Management Plan (RARMP) for limited and controlled release of wheat and barley genetically modified for enhanced nutrient utilization efficiency; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir094
OGTR (2009b) DIR 095/2009 - Full Risk Assessment and Risk Management Plan (RARMP) for limited and controlled release of sugarcane genetically modified for altered plant growth, enhanced drought tolerance, enhanced nitrogen use efficiency, altered sucrose accumulation, and improved cellulosic ethanol production from sugarcane biomass; http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/dir095
Oh S.J., Song S.I., Kim Y.S., Jang H.J., Kim S.Y., Kim M., Kim Y.K., Nahm B.H. and Kim J.K. (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiology 138: 341-351.
Oraby H.F., Ransom C.B., Kravchenko A.N. and Sticklen M.B. (2005) Barley HVA1 gene confers salt tolerance in R3 transgenic oat. Crop Sci 45: 2218-2227.
Orr H.A. (2009) Fitness and its role in evolutionary genetics. Nature Reviews Genetics 10, 531-539.
Palenchar P.M., Kouranov A., Lejay L.V., Coruzzi G.M. (2004) Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants. Genome Biol. 5(11) R91
Park S., Li J., Pittman J.K., Berkowitz G.A., Yang H., Undurraga S., Morris J., Hirschi K.D. and Gaxiola R.A. (2005) Up-regulation of a H+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. PNAS 102(52): 18830-18835.
Parker I.M. and Kareiva P. (1996) Assessing the risks of invasion for genetically engineered plants: acceptable evidence and reasonable doubt. Biol. Conserv. 78: 193–203.
Paul M.J., Primavesi L.F., Jhurreea D. and Zhang Y. (2008) Trehalose Metabolism and Signaling. Annual Review of Plant Biology 59: 417-441.
Peña L., Martín-Trillo M., Juárez J., Pina J.A., Navarro L. and Martínez-Zapater J.M. (2001) Constitutive expression of *Arabidopsis* LEAFY or APETALA1 genes in citrus reduces their generation time. Nat. Biotech. 19: 263–267.
Peng J., Richards D.E., Hartley N.M., Murphy G.P, Devos K.M., Flintham J.E., Beales J., Fish L.J., Worland A.J., Pelica F., Sudhakar D., Christou P., Snape J.W., Gale M.D. and N.P. Harberd (1999) 'Green Revolution' genes encode mutant giberellin response modulators. Nature 400: 256–261.

Peterhänsel C., Niessen M. and Kebeish R.M. (2008) Metabolic Engineering Towards the Enhancement of Photosynthesis. Photochemistry and Photobiology 84(6): 1317 – 1323.
Pierik R., Cuppens M.L.C., Voesenek L.A.C.J. and Visser E.J.W. (2004) Interactions between Ethylene and Gibberellins in Phytochrome-Mediated Shade Avoidance Responses in Tobacco. Plant Physiol., 136: 2928–2936.
Pimenta Lange M.J. and Lange T. (2006) Gibberellin Biosynthesis and the Regulation of Plant Development. Plant Biol. 8(3): 281–290.
Poppy G.M. and Wilkinson M.J. (2005) Gene Flow from GM Crops. Oxford, UK: Blackwell. 241 pp.
Pretty J. (2001) The rapid emergence of genetic modification in world agriculture: contested risks and benefits. Environmental Conservation 28: 248-262.
Radosevich S.R. and Holt J.S. (1984) Weed Ecology: Implications for Vegetation Management (265 pp.) New York: John Wiley & Sons.
Ragauskas A.J., Williams C.K., Davison B.H., Britovsek G., Cairney J., Eckert C.A., Frederick Jr. W.J., Hallett J.P., Leak D.J., Liotta C.L., Mielenz J.R., Murphy R., Templer R., Tschaplinski T. (2006) The Path Forward for Biofuels and Biomaterials. Science 311: 484–489.
Raybould A. (2006) Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ Biosafety Res 5: 119–125.
Richards R.A. (1996) Defining selection criteria to improve yield under drought Plant Growth Regulation 20(2): 157-166.
Rieseberg L.H. and Burke J.M. (2001) The biological reality of species: gene flow, selection, and collective evolution. Taxon 50: 47–67.
Riewe D., Grosman L., Fernie A.R., Wucke C. and Geigenberger P. (2008) The Potato-Specific Apyrase Is Apoplastically Localized and Has Influence on Gene Expression, Growth and Development. Plant Physiol. 147: 1092–1109.
Roelofs D., Aarts M.G.M., Schat H. and van Straalen N.M. (2008) Functional ecological genomics to demonstrate general and specific responses to abiotic stress. Funct. Ecol. 22: 8–18.
Rolland F., Baena-Gonzalez E. and Sheen J. (2006) Sugar sensing and signaling in plants: Conserved and Novel Mechanisms. Annu. Rev. of Plant Biol. 57: 675-709.
Rothfels C.J., Beaton L.L. and Dudley S.A. (2002) The effects of salt, manganese and density on life history traits in *Hesperis matronalis* from oldfield and roadside populations. Can. J. Bot. 80: 131–139.
Roxas V.P., Smith R.K. Jr, Allen E.R. and Allen R.D. (1997) Over-expression of glutathione S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotech 15: 988–991.
Rueda E.C., Dezar C.A., Gonzalez D.H., and Chan R.L. (2005) *Hahb-10*, a Sunflower Homeobox-Leucine Zipper Gene, Is Regulated by Light Quality and Quantity and Promotes Early Flowering when Expressed in *Arabidopsis*. Plant and Cell Physiology 46: 1954–1963.
Saibo N.J.M., Lourenco T. and Oliveira M.M. (2009) Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. Ann. Bot. 103(4): 609–623.
Sakakibara H. (2006) CYTOKININS: Activity, Biosynthesis, and Translocation. Annu Rev. of Plant Biol. 57: 431-449.
Sakakibara H., Takei K. and Hirose N. (2006) Interactions between nitrogen and cytokinin in the regulation of metabolism and development Trends in Plant Science 11(9): 440-448.
Sakamoto A. and Murata N. (2000) Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. J. Exp. Botany 51(342): 81–88.
Sakamoto A. and Murata N. (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant, Cell and Environment 25: 163–171.
Sakamoto T., Morinaka Y., Ishiyama K., Kobayashi M., Itoh H., Kayano T., Iwahori S., Matsuoka M., and Tanaka H. (2003) Genetic manipulation of gibberellin metabolism in transgenic rice. Nature Biotechnology 21: 909–913.
Salas Fernandez M.G., Becraft P.W., Yin Y. and Lübberstedt T. (2009) From dwarves to giants? Plant height manipulation for biomass yield. Trends in Plant Sci 14(8): 454-461.
Salehi H., Ransom C.B., Oraby H.F., Seddighi Z. and Sticklen M.B. (2005) Delay in flowering and increase in biomass of transgenic tobacco expressing the *Arabidopsis* floral repressor gene FLOWERING LOCUS C. J. Plant Physiol. 162: 711–717.
Sanmiya K., Suzuki K., Egawa Y. and Shono M. (2004) Mitochondrial small heat-shock protein enhances thermotolerance in tobacco plants. FEBS Lett. 557(1-3): 265-268.

Schenkelaars Biotechnology Consultancy (2007) Novel aspects of the environmental risk assessment of drought-tolerant genetically modified maize and omega-3 fatty acid genetically modified soybean. (last consulted 15 December 2009, http://www.sbcbiotech.nl/page/downloads/Report_DT-GM_maize__O-3-FA-GM-soybean_final_version_31.10.2007.pdf)

Schwechheimer C. and Willige B.C. (2009) Shedding light on gibberellic acid signaling. Curr. Opin. Plant Biol. 12: 57-62.

Schwechheimer C., Zourelidou M. and Bevan M.W. (1998) Plant transcription factor studies. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 127-150.

Shen B., Jensen R.G. and Bohnert H.J. (1997) Mannitol protects against oxidation by hydroxyl radicals. Plant Physiol 115: 1211-1219.

Shinozaki K. and Yamaguchi-Shinozaki K. (2007) Gene networks involved in drought stress response and tolerance. J. Exp. Bot. 58(2): 221–227.

Shkolnik D. and Bar-Zvi D. (2008) Tomato ASR1 abrogates the response to abscisic acid and glucose in *Arabidopsis* by competing with ABI4 for DNA binding. Plant Biotechnology Journal 6(4): 368-378.

Shrawat A.K. and Good A.G. (2008) Genetic Engineering Approaches to Improving Nitrogen Use Efficiency. ISB news report, May 2008.

Siegal M.L. and Bergmann A. (2002) Waddington's canalization revisited: Developmental stability and evolution Proc. Natl. Acad. Sci. U.S.A. 99: 10528.

Silverstone A.L. and Sun T. (2000) Gibberellins and the Green Revolution. Trends in Plant Science 5(1): 1–2.

Sinclair T.R., Purcell L.C., Sneller C.H. (2004) Crop transformation and the challenge to increase yield potential. Trends in Plant Science 9: 70–75.

Singla-Pareek S.L., Reddy M.K. and Sopory S.K. (2003) Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. PNAS 100: 14672–14677.

Singla-Pareek S.L., Yadav S.K., Pareek A., Reddy M.K., Sopory S.K. (2008) Transgenic Res. 17(2): 171-180.

Sivamani E., Bahieldin A., Wraith J.M., Al-Niemi T., Dyer W.E., Ho T.H.D. and Wu R. (2000) Improved biomass productivity and water use efficiency under water-deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. Plant Sci 155: 1–9.

Slatkin M. (1987) Gene flow at the geographic structure of natural populations. Science 236: 787–792.

Small I. (2007). RNAi for revealing and engineering plant gene functions. Current Opinion in Biotechnology 18: 148-153.

Smeekens S. (2000) Sugar-induced signal transduction in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51: 49-81.

Smidansky E.D., Martin J.M., Hannah L.C., Fischer A.M. and Giroux M.J. (2003) Seed yield and plant biomass increases in rice are conferred by deregulation of endosperm ADP-glucose pyrophosphorylase. Planta 216: 656–664.

Smidansky E.D., Clancy M., Meyer F.D., Lanning S.P., Blake N.K., Talbert L.E. and Giroux M.J. (2002) Enhanced ADP-glucose pyrophosphorylase activity in wheat endosperm increases seed yield. PNAS 99: 1724-1729.

Snow A.A., Andow D.A., Gepts P., Hallerman E.M., Power A., Tiedje J.M. and Wolfenbarger L.L. (2005) Genetically Engineered Organisms And The Environment: Current Status And Recommendations Ecological Applications 15(2): 377–404.

Snow A.A., Pilson D., Rieseberg L.H., Paulsen M.J., Pleskac N., Reagon M.R., Wolf D.E. and Selbo S.M. (2003) A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol. Appl. 13: 279–286.

Spangenberg G. (2008) Drought-tolerant wheat: "Promising results"; http://www.gmo-safety.eu/en/news/654.docu.html (last accessed 20/12/2009)

Srivalli S. and Khanna-Chopra R. (2008) Role of Glutathione in Abiotic Stress Tolerance. In, Sulfur Assimilation and Abiotic Stress in Plants, Khan N.A. Singh S., Umar S. (eds.) Springer-Verlag Berlin Heidelberg, p. 207-225.

Stein A.J. and Rodríguez-Cerezo E. (2009) The global pipeline of new GM crops: Implications of asynchronous approval for international trade. JRC Scientific and Technical Reports. JRC51799, EUR 23846 EN, 114p.

Stiller I., Dulai S., Kondrák M., Tarnai R., Szabó L., Toldi O. and Bánfaldi Z. (2008) Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae*. Planta 227(2): 299-308.

Stitt M. (1999) Nitrate regulation of metabolism and growth. Curr. Opin. Plant Biol. 2: 178–186.

Strange A., Park J., Bennett R., Phipps R. (2008) The use of life-cycle assessment to evaluate the environmental impacts of growing genetically modified, nitrogen use-efficient canola. Plant Biotechnology Journal 6(4): 337-345.

Strauss S.H. (2003) Genomics, Genetic Engineering and Domestication of Crops. Science 300: 61-62.

Strauss S.H. (2005) Genetic engineering approaches to breeding sterility and reduced invasiveness (presentation) (last consulted 15 December 2009) www.ars.usda.gov/sp2UserFiles/Program/301/FNRIDocs/2005/Day1/Strauss%20-%20Floral%20and%20Nursery%20Crops%20Researchers%20Workshop%20No%202.ppt

Strauss S.H. (2006) Development & validation of sterility systems in trees (Reports on Completed R&D Projects by the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy), http://www1.eere.energy.gov/industry/forest/pdfs/strauss.pdf (last consulted 15 December 2009)

Sunkar R., Bartels D. and Kirch H.H. (2003) Overexpression of a stressinducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. Plant J. 35: 452-464.

Taguchi-Shiobara F., Yuan Z., Hake S. and Jackson D. (2001) The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. Genes & Development 15: 2755–2766.

Taniguchi Y., Ohkawa H., Masumoto C., Fukuda T., Tamai T., Lee K., Sudoh S., Tsuchida H., Sasaki H., Fukayama H. and Miyao M. (2008) Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: an approach to introduce the C_4-like photosynthetic pathway into rice. J. Exp. Bot. 59(7): 1799-1809.

Tester M. and Bacic A. (2005) Abiotic Stress Tolerance in Grasses. From Model Plants to Crop Plants Plant Physiology 137: 791–793.

Van Dijken A.J., Schluepmann H. and Smeekens S.C. (2004) *Arabidopsis* trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. Plant Physiol. 135: 969–977.

Vasconcelos M., Eckert H.,·Arahana V., Graef G., Grusak M.A. and·Clemente T. (2006) Molecular and phenotypic characterization of transgenic soybean expressing the *Arabidopsis* ferric chelate reductase gene, FRO2. Planta 224: 1116–1128.

Vasil I.K. (2007) Molecular genetic improvement of cereals: transgenic wheat (*Triticum aestivum* L.). Plant Cell Rep 26: 1133–1154.

Vij S. and Tyagi A. (2008) A20/AN1 zinc-finger domain-containing proteins in plants and animals represent common elements in stress response. Functional & Integrative Genomics 8(3): 301-307.

Vinocur B. and Altman A. (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology 16: 123–132.

Vitousek P.M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. Oikos 57: 7–13.

von Groll U., Berger, D. and Altmann T. (2002) The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during *Arabidopsis* stomatal development. Plant Cell 14: 1527-1539.

Wang W., Vinocur B. and Altman A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218: 1–14.

Wang W., Vinocur B. Shoseyov O. and Altman A. (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in Plant Science 9(5): 244-252.

Wang Y., Beaith M., Chalifoux M., Ying J., Uchacz T., Sarvas C., Griffiths R., Kuzma M., Wan J. and Huang Y. (2009) Shoot-Specific Down-Regulation of Protein Farnesyltransferase (alpha-Subunit) for Yield Protection against Drought in Canola. Mol. Plant 2(1): 191-200.

Wang Y., Ying J., Kuzma M., Chalifoux M., Sample A., McArthur C., Uchacz T., Sarvas C., Wan J., Dennis D.T., McCourt P. and Huang Y. (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. The Plant Journal 43: 413–424.

Warwick S.I. and Stewart Jr. C.N. (2005) Crops come from wild plants—how domestication, transgenes and linkage together shape ferality. In J. Gressel (Ed.), Crop Ferality and Volunteerism (pp. 9–30). London: CRC Press.

Warwick S.I., Beckie H.J. and Hall L.M. (2009) Gene Flow, Invasiveness, and Ecological Impact of Genetically Modified Crops The Year in Evolutionary Biology 2009: Ann. N.Y. Acad. Sci. 1168: 72–99.

Waterhouse P.M. and Helliwell C.A. (2003) Exploring plant genomes by RNA-induced gene silencing. Nature Reviews: Genetics 4: 29-38.

Weis A.E. (2005) Assessing the ecological fitness of recipients. In G.M. Poppy and M.J. Wilkinson (Eds.), Gene Flow from GM Plants (pp. 143–168). Oxford, UK: Blackwell Publishing.

Wilkinson M. and Tepfer M. (2009) Fitness and beyond: Preparing for the arrival of GM crops with ecologically important novel characters Environ. Biosafety Res. 8: 1–14.

Williamson M. (1994). Community response to transgenic plant release: Prediction from British experience of invasive plants and feral crop plants. Molecular Ecology 3: 75-79.

Wolfe L.M. and Blair A.C. (2007) Born to run: competition enhances the spread of genes from crops to wild relatives. New Phytol. 173: 450–452.

Wolt J.D., Keese P., Raybould A., Fitzpatrick J.W., Burachik M., Gray A., Olin S.S., Schiemann J., Sears M. and Wu F. (2009) Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Res DOI 10.1007/s11248-009-9321-9.

Wu C.Y., Trieu A., Radhakrishnan P., Kwok S.F., Harris S., Zhang K., Wang J., Wan J., Zhai H., Takatsuto S., Matsumoto S., Fujioka S., Feldmann K.A. and Pennella R.I. (2008) Brassinosteroids regulate grain filling in rice. Plant Cell 20: 2130–2145.

Wu W., Su Q., Xia X.Y., Wang Y., Luan Y.S. and An L.J. (2008) The *Suaeda liaotungensis* kitag betaine aldehyde dehydrogenase gene improves salt tolerance of transgenic maize mediated with minimum linear length of DNA fragment. Euphytica 159: 17-25.

Wu X., Shiroto Y., Kishitani S., Ito Y. and Toriyama K. (2009) Enhanced heat and drought tolerance in transgenic rice seedlings over-expressing OsWRKY11 under the control of HSP101 promoter. Plant Cell Rep 28: 21–30.

Xie Q., Guo H.-S., Dallman G., Fang S., Weissman A.M. and Chua N.-H. (2002) SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. Nature 41: 167-170.

Xu D., Duan X., Wang B., Hong B., Ho T.D. and Wu R. (1996) Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110: 249–257.

Yamaguchi S. (2008) Gibberellin Metabolism and its Regulation. Annu. Rev. Plant Biol. 59: 225-251.

Yamaguchi-Shinozaki K. and Shinozaki K. (2006) Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. Annu. Rev. Plant Biol. 57: 781-803.

Yamaya T., Obara M., Nakajima H., Sasaki S., Hayakawa T. and Sato T. (2002) Genetic manipulation and quantitative-trait loci mapping for nitrogen recycling in rice. J. Exp. Bot. 53: 917-925.

Yan L., Helguera M., Kato K., Fukuyama S., Sherman J. and Dubcovsky J. (2004) Allelic variation at the VRN-1 promoter region in polyploidy wheat. Theor. Appl. Genet. 109: 1677–1686.

Yan L., Loukoïanov A., Tranquilli G., Helguera M., Fahima T. and Dubcovsky J. (2003) Positional cloning of the wheat vernalization gene VRN1. PNAS 100(10): 6263-6268.

Yanagisawa S., Akiyama A., Kisaka H., Uchimiya H. and Miwa T. (2004) Metabolic engineering with Dof1 transcription factor in plants: Improved nitrogen assimilation and growth under low-nitrogen conditions. PNAS 101: 7833–7838.

Yang S.-M., Chang C.-Y., Yanagisawa M., Park I., Tseng T.-H. and Ku M.S.B. (2008) Transgenic rice expressing cyanobacterial bicarbonate transporter exhibited enhanced photosynthesis, growth and grain yield. In Photosynthesis. Energy from the Sun. 14th International Congress on Photosynthesis. Ed. Allen J.F., Gantt E., Golbeck J.H. and Osmond B. Springer Netherlands, 1243-1246.

Yoshioka K. and Shinozaki K. (Eds) (2009) Signal Cross-talk in Plant Stress Responses Wiley-Blackwell; ISBN: 978-0-8138-1963-1; 224 pages

Zhang L., Häusler R.E., Greiten C., Hajirezaei M.R., Haferkamp I., Neuhaus H.E., Flügge U.I. and Ludewig F. (2008) Overriding the co-limiting import of carbon and energy into tuber amyloplasts increases the starch content and yield of transgenic potato plants. Plant Biotechnol. J. 6(5): 453-464.

Zhao F.-Y. and Zhang H. (2007) Transgenic Rice Breeding for Abiotic Stress Tolerance - Present and Future, Chin J Biotech. 23(1): 1−6.

Zhou Q.-Y., Tian A.-G., Zou H.-F., Xie Z.-M., Lei G., Huang J., Wang C.-M., Wang H.-W., Zhang J.-S., and Chen S.-Y. (2008) Soybean WRKY-type transcription factor genes, GmWRKY13,

GmWRKY21, and GmWRKY54, confer differential tolerance to abiotic stresses in transgenic *Arabidopsis* plants. Plant Biotechnol. J. 6(5): 486–503.

Zhu G., Kurek I., True T., Zhang X., Majumdar M., Liu L. and Lassner M. (2004) Enhancing photosynthesis by improving Rubisco carboxylase activity and specificity, and Rubisco activase thermostability through DNA shuffling. In Photosynthesis: Fundamental Aspects to Global Perspectives. Proceedings of the 13th International Congress on Photosynthesis. Montreal, Canada; Editors A. van der Est and D. Bruce.

7 References – Patents

Applicant	Reference	Subject	Publication date
Agrigenesis Biosciences LTD (New-Zealand)	WO 2005-010191A1	Plant Cell Cycle Genes And Methods Of Use	February 3, 2005.
Ajinomoto Co., Inc.	US Patent 7,176,351	Potatoes Having An Increased Yield Of Starch Per Plant Body And Method For Producing The Same	February 13, 2007.
ArborGen	US patent 7,371,927	Methods For Modulating Plant Growth And Biomass	May 13, 2008
ArborGen	US patent 7,598,084	Modifications Of Plant Traits Using Cyclin A	October 6, 2009.
ArborGen	WO 2008/121320A3	Enhancement Of Stress Tolerance In Plants	December 18, 2008.
Arcadia	WO 2007/076115	Nitrogen-Efficient Monocot Plants	July 5, 2007.
BASF Plant Science	WO 2001/014569	Increasing The Polysaccharide Content In Plants	March 1, 2001.
BASF Plant Science	WO 2002/016423	Plant Polynucleatides encoding novel Na^+/H^+ antiporters	February 28, 2002
BASF Plant Science	WO 2003/077643	Methods For Increasing The Oil Content In Plants By Reducing The Content Of One Or More Storage Proteins	September 26, 2003.
BASF Plant Science	WO 2003/095655	Methods For Increasing Oil Content In Plants	November 11, 2003.
BASF Plant Science	WO 2004/007727	Use Of A Gene For Increasing The Oil Content In Plants	January 22, 2004
BASF Plant Science	WO 2008/059048	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same Using Consensus Sequences From The Yabby Protein Family	May 22, 2008.
BASF Plant Science	WO 2009/013263	Plants Having Increased Yield-Related Traits And A Method For Making The Same	January 29, 2009.
BASF Plant Science	WO 2009/016212	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same	February 5, 2009
BASF Plant Science	WO 2009/016232	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same	February 5, 2009
BASF Plant Science	WO 2009/027335	Polypeptides, Such As Lipases, Capable Of Altering The Seed Storage Content In Transgenic Plants	March 5, 2009.
BASF Plant Science	WO 2009/037279	Plants With Increased Yield	March 26, 2009
BASF Plant Science	WO 2009/056566	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same	May 7, 2009.
BASF Plant Science	WO 2009/080743	Plants With Increased Yield (Ko Nue	July 2, 2009.
BASF Plant Science	WO 2009/095455	Plants Having Increased Yield-Related Traits And A Method For Making The Same	August 6, 2009.
Bayer BioScience N.V.	WO/2004/090140	Methods And Means For Increasing The Tolerance Of Plants To Stress Conditions	October 21, 2004.
Bayer BioScience N.V.	WO/2006/032469	Stress Resistant Plants	March 30, 2006.
Bayer BioScience N.V.	WO/2007/107326	Stress Resistant Plants	September 27, 2007.
BIOCERES S.A.	Argentinean patent application AR039518	Gen De Un Factor De Transcripcion Inducible Por Condiciones De Estres	February 23, 2005.

	A1	Hidrico Y Acido Abscisico De Helianthus Annuus, Promotor Y Plantas Transgenicas	
BIOCERES S.A.	Argentinean patent application AR053194 A1	Construcciones De ADN Que Contienen La Secuencia Codificante Del Gen Hahb-10 De Helianthus Annuus, Metodo Para Generar Plantas Con El Ciclo De Vida Acortado Y Con Alta Tolerancia A Herbicidas Y Plantas Transgenicas Con Dicha Secuencia	April 25, 2007 .
BIOCERES S.A.	US patent application 20070180584	Transcription Factor Gene Induced By Water Deficit Conditions And Abscisic Acid From Helianthus Annuus, Promoter And Transgenic Plants	August 2, 2007.
BIOCERES S.A.	US patent application 20070234439	DNA Constructs That Contain Helianthus Annuus Hahb-10 Gene Coding Sequence, Method For Generating Plants With A Shortened Life Cycle And A High Tolerance To Herbicidal Compounds And Transgenic Plants With That Sequence	October 4, 2007.
Biogemma	WO 2001/083756	Method For Obtaining Plants Exhibiting Enhanced Resistance To Water Stress	November 8, 2001
Biogemma	WO 2002/081714	Over-Expression Of Phosphoenolpyruvate Carboxylase	October 17, 2002.
Biogemma	WO 2003/074713	Use Of Early Light-Inducible Proteins (Elip To Increase Plant Resistance To Photochemical Oxidant Stress)	September 12, 2003.
Biogemma	WO 2007/093623	Zmtcrr-1 Plant Signal Transduction Gene And Promoter	August 23, 2007.
Biogemma UK Ltd.	WO 1999/015680	Control Of Plant Abscission And Pod Dehiscence Or Shatter	April 1, 1999.
Biogemma UK Ltd.	WO 1999/015681	Control Pop Dehiscence Or Shatter	April 1, 1999.
Ceres Inc.	WO 2009/102965	Drought And Heat Tolerance In Plants	August 20, 2009.
Ceres Inc.	WO 2009/105492	Transgenic Plants Having Altered Nitrogen Use Efficiency Characteristics	August 27, 2009.
Ceres Inc.	WO 2009/105612	Nucleotide Sequences And Corresponding Polypeptides Conferring Improved Nitrogen Use Efficiency Characteristics In Plants	August 27, 2009.
Ceres Inc.	WO 2009/114733	Nucleotide Sequences And Corresponding Polypeptides Conferring Modulated Growth Rate And Biomass In Plants Grown In Saline And Oxidative Conditions	September 17, 2009.
CropDesign	WO 2000/056905	Method For Enhancing And/Or Improving Plant Growth And/Or Yield Or Modifying Plant Architecture	September 28, 2000.
CropDesign	WO 2001/031041	Modification Of Plant Architecture	May 3, 2001.
CropDesign	WO 2001/096579	A Novel Plant Cyclin	December 10, 2001.
CropDesign	WO 2003/025185	A Method To Modify Cell Number, Architecture And Yield Of Plants By Over-Expressing The E2f Transcription Factor	March 27, 2003.
CropDesign	WO 2003/027299	A Plant Cyclin Dependent Kinase-Like Protein, Its Interactors And Uses Thereof	April 3, 2003.
CropDesign	WO 2004/016775	Plants Having Modified Growth And A Method For Making The Same	February 26, 2004.
CropDesign	WO 2004/029257	Plants Having Changed Development And A Method For Making The Same	April 8, 2004.
CropDesign	WO 2004/058980	Plants Having Modified Growth Characteristics And A Method For Making	July 15, 2004.

		The Same	
CropDesign	WO 2004/070027	Method For Modifying Plant Growth Characteristics	August 19, 2004.
CropDesign	WO 2005/024029	Plants Having Modified Growth Characteristics And Method For Making The Same	March 17, 2005.
CropDesign	WO 2005/061702	Plants Having Increased Yield And Method For Making The Same	July 7, 2005.
CropDesign	WO 2005/083094	Plants Having Increased Yield And Method For Making The Same	September 9, 2005.
CropDesign	WO 2005/085452	Plants Having Improved Yield And Method For Making The Same	September 15, 2005.
CropDesign	WO 2005/117568	Plants Having Improved Growth Characteristics And A Method For Making The Same	December 15, 2005.
CropDesign	WO 2006/045829	Plants Having Improved Growth Characteristics And A Method For Making The Same	May 4, 2006
CropDesign	WO 2006/056590	Plants Having Increased Yield And A Method For Making The Same.	June 1, 2006
CropDesign	WO 2006/058897	Plants Having Improved Growth Characteristics And Method For Making The Same	June 8, 2006.
CropDesign	WO 2006/079655	Plants Having Increased Yield And A Method For Making The Same	August 3, 2006.
CropDesign	WO 2006/100112	Plants Having Increased Yield And A Method For Making The Same	September 28, 2006.
CropDesign	WO 2007/003660	Plant Yield Improvement By Ste20-Like Gene Expression	January 11, 2007.
CropDesign	WO 2007/051866	Plants Having Improved Growth Characteristics And A Method For Making The Same	June 10, 2007.
CropDesign	WO 2007/064724	Plants Having Improved Growth Characteristics And Methods For Making The Same	June 7, 2007.
CropDesign	WO 2008/071767	Plants Having Enhanced Seed Yield-Related Traits And A Method For Making The Same	June 19, 2008.
CropDesign	WO 2008/092910	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same	August 7, 2008.
CropDesign	WO 2008/132231	Plants Having Improved Growth Characteristics Under Reduced Nutrient Availability And A Method For Making The Same	November 6, 2008.
CropDesign	WO 2008/148896	Yield Enhancement In Plants By Modulation Of Maize Mads Box Transcription Factor Silky1	December 11, 2008.
CropDesign	WO 2008/148872	Yield Enhancement In Plants By Modulation Of Maize Mads Box Transcription Factor Zmm28	December 11, 2008.
CropDesign	WO 2009/013225	Plants Having Enhanced Yield-Related Traits And A Method For Making The Same	January 29, 2009.
CropDesign	WO 2009/065912	Plants Having Increased Yield-Related Traits And A Method For Making The Same	May 28, 2009.
DuPont de Nemours and Company	US Patent 6,812,382	Plant Genes Encoding Chloroplast Division Proteins	November 2, 2004.

DuPont de Nemours and Company & Cold Spring Harbor laboratory	WO 2006/074437	Nucleotide Sequences Encoding Ramosa3 And Sister Of Ramosa3 And Methods Of Use For Same	July, 13, 2006.
DuPont de Nemours and Company	US Patent Application 20090172842	Expression Of Recombinant Genes Encoding Rubisco Proteins In C3 Plants	February 7, 2009
La Trobe University	US Patent 7,227,055	Manipulation Of Plant Senescence	June 5, 2007.
Mendel Biotechnology	WO 2004/076638	Polynucleotides And Polypeptides In Plants	September 10, 2004.
Mendel Biotechnology	WO/2005/030966	Regulation Of Plant Biomass And Stress Tolerance	April 7, 2005
Mendel Biotechnology	WO 2005/047516	Plant Transcriptional Regulators	May 26, 2005.
Mendel Biotechnology	US Patent Application 20090265813	Stress Tolerance In Plants	October 22, 2009.
Monsanto Technology LLC	US Patent 6,663,906	Expression Of Fructose 1,6 Bisphosphate Aldolase In Transgenic Plants	December 16, 2003.
Monsanto Company	US patent 2006/236419 A1	Nucleic Acid Molecules And Other Molecules Associated With Plants And Uses Thereof For Plant Improvement	October 19, 2006.
Monsanto Company	United States Patent Application 20080104730	Yield-Improved Transgenic Plants	May 1, 2008.
Performance Plants Inc.	WO 99/006580	Stress Tolerance And Delayed Senescence In Plants	February 11, 1999.
Performance Plants Inc.	WO 2004/020642	Stress Tolerance And Delayed Senescence In Plants	March 11, 2004.
Performance Plants Inc.	WO 2009/027824	Plants Having Increased Tolerance To Heat Stress	March 5, 2009.
Performance Plants Inc.	WO 2009/040665	Plants Having Increased Biomass	April 2, 2009.
Pioneer HI-Bred International, Inc.	WO 2004/063379	Zea Mays Revoluta/If1 Homolog Genes And Uses Thereof	July 29, 2004.
Pioneer Hi-Bred International, Inc.	US patent 7,179,963	Maize CLAVATA3-Like Polynucleotide Sequences And Methods Of Use	February 20, 2007.
Plant Genetic Systems	WO 1997/013865	Seed Shattering	April 17, 1997.
Targeted Growth	WO 2007/016319	Dominant Negative Mutant Krp Protein Protection Of Active Cyclin-Cdk Complex Inhibition By Wild-Type Krp	February 8, 2007.
Targeted Growth	WO 2007/079353	Increased Seed Size And Seed Number Through Transgenic Over Expression Of A Growth And/Or Development Related Gene During Early Embryo Development	December 15, 2006.
University of Nevada	US patent 5,981,836	Plant Plastid Division Genes	November 9, 1999.
VIB, KULeuven Research and Development	WO 2007/085483	Use Of Trehalose-6-Phosphate Synthase To Modulate Plant Growth	August 2, 2007.
University of Connecticut, Whithead Institute for biomedical research, Beth Israel Deaconess Medical Center	WO 2009/020528	Vacuolar Pyrophosphatases And Uses In Plants	February 12, 2009.
University of Alberta	US patent 6,084,153	Plants Having Enhanced Nitrogen Assimilation/Metabolism	July 4, 2000.

Annex 1 Selected GMO Field trial applications in the EU

Source: Deliberate release and placing on the market of Genetically Modified Organisms - GMO register managed by the Joint Research Centre of the European Commission (last accessed 21 December 2009, http://gmoinfo.jrc.ec.europa.eu/)

Application number	crop	trait	Institute/company	genes
B/DE/92/05	potato	Increased yield	Institut für Genbiologische Forschung Berlin GmbH	Invertase (SUC2) from *Saccharomyces cerevisiae*
B/ES/94/10	sunflower	Drought tolerance	Van der Have Cubian SA	
B/DE/96/47	potato	Increased yield	Max-Planck-Institut für Molekulare Pflanzenphysiologie	Polyphosphate kinase (PPK) *E. coli;* Sucrosetransporter (SoSUT) *Spinacia oleracea;* Hexosephosphate Translokator (UhpT) *E. coli*; mitochondrial PhosphateTransporter (PTP) *S. cerevisiae;* PhosphateTransporter (Pho84) *S. cerevisiae*, Invertase (SUC2) *S. cerevisiae*
B/FR/96/02/13 B/FR/96/02/15	sugar beet	Drought tolerance	Van der Have France	
B/IT/96/50/A	tomato	Increased yield	Sementi Nunhems Srl	sucrose transporter protein
B/IT/96/50/B	tomato	Increased yield	Sementi Nunhems Srl	polyphosphate kinase
B/IT/96/50/C	tomato	Increased yield	Sementi Nunhems Srl	synthesis of sucrose phosphate
B/IT/96/50/E	tomato	Drought tolerance	Sementi Nunhems Srl	
B/NL/96/13 B/NL/97/03 B/NL/97/03-EXT1	maize	Stress tolerance	D.J. van der Have	
B/FR/97/05/06 B/FR/97/05/06-EXT	maize	Stress tolerance	COOP de Pau	superoxide dismutase
B/IT/97/14	potato	Drought tolerance	Istituto per l'Agrometereologia e l'Analisi Ambientale Applicata all'Agricoltura IATA - CNR	
B/DE/98/87	potato	Increased yield	Max-Planck-Institut für Molekulare Pflanzenphysiologie	Polyphosphate kinase (PPK) *E. coli;* Citrata Synthase (mCS) *S. Cerevisiae*; Starch phosphorylase (Pho2) *S. tuberosum*; GDP-Mannose-Pyrophosphorylase (MPPY) *S. tuberosum*; Sucrosetransporter (SoSUT) *Spinacia oleracea;*

				H^+-ATPase (D PMA1) *S. cerevisiae*; (D PHA2) *S. tuberosum*; Sucrose synthae (SuSy) *S. tuberosum*
B/ES/98/07	tomato	Increased yield	Nestle R&D Center SA	
B/ES/98/27	poplar alba x tremula	Stimulation of growth rate synthesis of glutamine synthetase testing of gene stability	Universidad de Malaga Instituto Andaluz de Biotecnología Laboratorio de Bioquímica y Biología Molecular, Facultad de Ciencias	glutamine synthetase GS
B/IT/99/22	watermelon	Increased yield	Sementi Nunhems Srl	
B/DE/00/127	potato	Stress tolerance	MPI für Züchtungsforschung	
B/FR/00/03/02	maize	Stress tolerance	Biogemma	
B/FR/01/03/04 B/FR/01/03/06	maize	Drought tolerance	Biogemma	
B/BE/99/VW3, B/BE/00/V5 B/BE/01/V5B/BE/02/VW4	oilseed rape	Silique shatter resistance	Bayer	
B/ES/02/16	wheat	Improvement of the efficiency of ammonium assimilation/retention synthesis of glutamine synthetase	Institut de Recerca i Tecnologia Agroalimentàries IRTA	glutamine synthetase GS
B/ES/03/15-CON to B/ES/03/33-CON	rice	Increased yield, abiotic stress tolerance	CropDesign	19 genes CBI
B/DE/04/157	potato	Increased yield	Max-Planck-Institute of Molecular Plant Physiology	*apyrase 1* *Solanum tuberosum*
B/DE/04/159 B/DE/05/167	potato	Water use efficiency	Max-Planck-Institute of Molecular Plant Physiology	subtilisin like serine protease SDD1 gene *Solanum tuberosum*
B/SE/04/1309	*Populus tremula x tremuloides*	Study photosynthetic genome	Department of Plant Physiology, Umeå University	*PsbS* gene (RNAi)
B/SE/04/1310	*Arabidopsis*	Study photosynthetic genome	Department of Plant Physiology, Umeå University	photosynthetic genes (knock-outs)
B/DE/05/175	potato	Increased yield	University of Cologne	*gpt* - glucose-6-phosphate/phosphate translocator *Pisum sativum*; *ntt1* - adenylate translocator 1 *A. thaliana*
B/FR/03/02/04 B/FR/05/01/01	maize	Nitrogen use efficiency	Biogemma	*Gs-1b* *Gln1-3* - glutamine synthetase *Zea mays*
B/FR/05/02/02	maize	Drought tolerance	Biogemma	*asr1* - abscisic acid stress ripening *Zea mays*
B/FR/06/12/06 B/FR/06/01/13 B/FR/05/02/03 B/FR/03/03/04	maize	Increased photosynthesis Drought tolerance	Biogemma	*Pepc* –phosphoenolpyruvate carboxylase *Sorghum bicolor*

B/FR/06/02/03 B/FR/06/12/05	maize	Early flowering Increased yield	Biogemma	5 Transcription factor genes *Zea mays*
B/ES/06/43 B/ES/08/03	carrizo citrange	Modification of plant architecture, flowering and fruiting behaviour	Instituto Valenciano de Investigaciones Agrarias	over-expressing a GA20 oxidase gene in antisense: *CcGA 20-oxi1*
B/SE/06/724 withdrawn	spring oilseed rape	Increased yield	Plant Science Sweden AB	
B/HU/07/06	potato	Drought tolerance	Agricultural Biotechnology Center	*Tps1* - trehalose-6-phosphate synthase gene yeast
B/ES/08/05	sweet orange	Induction of early flowering stimulation of growth rate	Instituto Valenciano de Investigaciones Agrarias	over-expression of the floral meristem identity gene APETALA 1
B/SE/08/2142	*Arabidopsis*	Study photosynthetic genome	Department of Plant Physiology, Umeå University	*PsbS* gene (overexpression)
B/SE/09/2058	*Arabidopsis*	Study photosynthetic genome	Umeå University	Knock-out, ELIP early light-induced proteins
B/ES/09/57 B/ES/10/14	potato	Heat stress	Centro Nacional de Biotecnología- CSIC	gene promoting tuberization *Solanum tuberosum*
B/SE/09/12395	hybrid aspen	Increased growth	Umeå University	LRR protein; GRAS TF; PSS; KNOTTED-like homeobox TF 6, 7, 8 and 9; several WRKY TFs; bHLH TF; probably a regulator of the gibberellin response; HD-GLABRA2 TF and SET protein (*Populus*) and an *Arabidopsis* GA20oxidase

Annex 2 Selected applications for GMO field trials in Australia

Source: Record of GMOs and GM Product Dealings from the Office of the Gene Technology Regulator within the Australian Government Department of Health and Ageing (last accessed 1 February 2010, http://www.health.gov.au/internet/ogtr/publishing.nsf/Content/gmorec-index-1)

Application number	crop	trait	Institute/company	genes
DIR 053/2004	wheat	Salt tolerance	Grain Biotech Australia Pty Ltd	*δ-oat* - Ornithine aminotransferase *A. thaliana* Over-expression proline = osmoprotectant
DIR 061/2005	wheat	Salt tolerance	Grain Biotech Australia Pty Ltd	withdrawn
DIR 064/2006	cotton	Drought tolerance/water use efficiency	Monsanto	24 genes CBI
DIR 067/2006	cotton	Water logging tolerance	CSIRO	*AHb1* - non-symbiotic phytohaemoglobin *A. thaliana*
DIR 070/2006	sugarcane	Altered plant architecture, enhanced water or improved nitrogen use efficiency	BSES Ltd.	*MdS6PDH* D-sorbitol-6-phosphate dehydrogenase *Malus* x *domestica* *EcTPSP* fusion enzyme of a trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase *Escherichia coli* *AtMYB2* myeloblastosis interacting protein 2 *Arabidopsis thaliana* *ZmDOF1 Z. mays* DNA binding with one finger 1 *Zea mays*
DIR 071/2006	wheat	Drought tolerance	Victorian Department of Primary Industries	Confidential
DIR 077/2007	wheat/ barley	Drought tolerance Increased boron tolerance Increased abiotic stress tolerance	The University of Adelaide	*TADREB2* Dehydration responsive element binding protein 2 *T. aestivum* *TADREB3* Dehydration responsive element binding protein 3 *T. aestivum* *Bot1* - Boron transporter *H. vulgare* Transcription factor CBI *T. aestivum*
DIR 080/2007	wheat	Drought tolerance	Victorian Department of Primary Industries	15 genes CBI

DIR 081/2007	cotton	Drought tolerance/water use efficiency	Monsanto	50 genes CBI
DIR 083/2007	cotton	water logging tolerance	CSIRO	*AHb1* - non-symbiotic phytohaemoglobin *A. thaliana* *Adh* – alcohol dehydrogenase *G. hirsutum* *Pdc2* – pyruvate decarboxylase *A. thaliana*
DIR 084/2008	Torenia	enhanced P uptake	Florigene Pty Ltd	*Phr1* phosphate starvation response regulator 1 *A. thaliana*
DIR 094/2009 DIR 099/2010	Wheat & Barley	enhanced nutrient utilization efficiency / NUE	CSIRO	*Me1* metabolic enzyme gene *Hordeum vulgare* CBI
DIR 095/2009	Sugarcane	Altered plant growth, enhanced drought tolerance, enhanced nitrogen use efficiency, altered sucrose accumulation, and improved cellulosic ethanol production from sugarcane biomass	BSES Limited	*PcGA2ox-1* derived from runner bean, *HvGA20ox-1* and *HvGA20ox-2* from barley, *OsTB1* from rice and *ShTB1* from sugarcane; *OsDREB1A,* from rice, and 2 other genes; *ZmDof1* from maize Sugarcane genes
DIR 100/2010	Wheat	Enhanced carbon assimilation in drought and heat prone environments	CSIRO	26 genes for enhanced carbon assimilation, grain weight, heat tolerance and/or WUE from wheat or barley
DIR 102/2010	Wheat & Barley	Abiotic stress	The University of Adelaide	Gene for salinity, drought and low phosphorus tolerance from *A. thaliana*; NUE gene from barley; 25 TF genes from wheat, maize and barley; 4 drought tolerance genes from wheat and maize; gene for Zn uptake, barley; 3 salinity tolerance genes, moss, *A. thaliana*, yeast.

Annex 3 Selected USDA applications for GMO field trials in USA

Source: **Field Test Release Applications in the U.S.**
Database Provided by APHIS Biotechnology Regulatory Services
http://www.isb.vt.edu/cfdocs/fieldtests1.cfm

In the following table field trial applications are summarized according to trait as indicated by the applicant. The data cover the period between June 16, 1987 and October 6, 2009. For the trait 'Yield enhancement' all applications that are somehow described as improving yield are counted. Traits that may lead to a yield increase, such as 'photosynthesis enhanced', 'dwarfing', 'seed size increase' etc. are not taken into account. Sometimes the application records mention 2 or more different traits and consequently figures overlap between columns. Company figures also include subsidiaries.
One application may contain several events, genes and gene combinations. Multiple trial sites are often included. In most of the cases the permits is valid for one year.

Table: Number of US field trial applications per trait, applicant and crop

	trait	Yield enhancement	Abiotic Stress tolerance	Drought tolerance WUE	Cold tolerance	Salt tolerance	Heat tolerance	NUE
Total		827	94	585	98	86	33	144
By company	Monsanto	516	33	364	34	19	17	67
	BASF	51	11	4	0	0	0	0
	Pioneer	107	3	34	0	0	3	47
	Bayer	7	4	0	0	0	0	0
	Syngenta	8	8	22	0	0	0	0
	Arcadia	0	0	3	0	12	0	18
	Arborgen	0	0	0	33	0	0	0
	Targeted Growth	44	0	0	0	0	0	0
	Rutgers University	0	0	34	0	37	2	0
	Biogemma	16	0	24	0	0	0	1
By crop	maize	468	52	406	22	9	13	83
	soybean	175	18	20	11	10	7	15
	rice	51	0	2	0	9	0	5
	oilseed rape	46	1	6	2	1	0	19
	cotton	19	17	44	1	2	3	0
	tomato	8	4	6	2	4	5	1
	potato	0	2	11	18	0	0	0
	wheat	31	0	12	0	0	1	8
	eucalyptus	0	0	0	33	0	0	0
	grasses	0	0	53	4	45	3	4

The next table only lists applications where at least one gene related to yield or stress has been disclosed. The most recent ones are given first. The records cover the period between June 16, 1987 and October 6, 2009.

Abbreviations: AP: agronomic properties; CBI: confidential business information; FR: fungal resistance; HT: herbicide tolerance; IR: insect resistance MG: marker gene; NR: nematode resistance; OO: other; PQ: product quality;

Table : US field trial applications disclosing genes of interest

APHIS #	Organism	Institution	Gene(s)	Phenotype(s)
09-267-103N	**Rapeseed**	Arcadia Biosciences	AlaAT - Donor: Hordeum vulgare CBI* - Donor: CBI NPTII* - Donor: Escherichia coli	AP - Nitrogen Utilization Efficiency Increase
09-223-101N	**Mouse-ear cress**	University of Missouri	mGFP - Donor: Aequorea victoria mCherry - Donor: Discosoma sp. truncated PHOT1 - Donor: Arabidopsis thaliana PHOT1 - Donor: Arabidopsis thaliana BAR - Donor: Streptomyces viridochromogenes hpt - Donor: Streptomyces hygroscopicus	MG - Red Fluorescent Protein (Mcherry) AP - Drought Tolerance Increased AP - Hyperphototropic HT - Basta Tolerant MG - Green Fluorescent Protein (Mgfp) HT - Hygromycin Resistant
09-159-102N	**Sweet potato**	United States Department of Agriculture/ Agricultur	AVP1 - Donor: Arabidopsis thaliana NPTII* - Donor: Escherichia coli	AP - Drought Tolerance Increased
09-146-102N	**Soybean**	University of Illinois	bar* - Donor: Streptomyces hygroscopicus ICTB - Donor: Synechococcus elongatus fructose-1,6-sedoheptulose-1,7 bisphosphatase - Donor: Synechoccus sp.	AP - Photosynthesis Enhanced
09-117-107n	**Corn**	Cold Spring Harbor Laboratory	cyan fluorescent protein - Donor: Aequorea victoria PINFORMED1 - Donor: Zea mays RAMOSA3 - Donor: Zea mays RESPONSE REGULATOR 1(RR1) - Donor: Zea mays RESPONSE REGULATOR 7(RR7) - Donor: Zea mays Red fluorescent protein - Donor: Discosoma sp. TONOPLAST INTRINSIC PROTEIN1 (TIP1) - Donor: Zea mays Transcription factor LhG4 - Donor: S. cerevisiae Yellow fluorescent protein (YFP) - Donor: A. victoria b-glucuronidase, GUS - Donor: Escherichia coli PHYTOENE SYNTHASE - Donor: Zea mays	AP - Floral Development Altered AP - Branching Decreased AP - Inflorescence Development Altered AP - Seed Number Increased MG - Visual Marker OO - Transactivated Expression OO - Epitope Tagged Gene

			red fluorescent protein - Donor: Discosoma sp. trehalase gene, treF - Donor: Escherichia coli yellow fluorescent protein - Donor: Aequorea victoria phosphinothricin acetyltransferase* - Donor: Streptomyces hygroscopicus PEROXIN11 - Donor: Zea mays Yellow fluorescent protein - Donor: Aequorea victoria FASCIATED EAR2 - Donor: Zea mays ABPHYL1 - Donor: Zea mays Alpha Tubulin - Donor: Zea mays CYTOKININ OXIDASE 1 - Donor: Zea mays CYTOKININ OXIDASE 2 - Donor: Zea mays Mutator B gene - Donor: Zea mays FASCIATED EAR2 gene-epitope tagged - Donor: Z. mays FASCIATED EAR2- epitope tagged - Donor: Zea mays FLOURY2 - Donor: Zea mays Fluorescent protein fusion - Donor: Zea mays and Aequorea victoria Glucocorticoid receptor - Donor: Rattus norvegicus HISTIDINE KINASE2 (HK2) - Donor: Zea mays Histidine phosphotransferase - Donor: Zea mays Histone H1 - Donor: Zea mays ISOPENTENYL TRANSFERASE 2 (IPT2) - Donor: Zea mays LhG4 synthetic transactivator - Donor: S. cerevisiae RAMOSA1 - Donor: Zea mays Cyclin D2 gene - Donor: Zea mays	
09-103-109n	**Peanut**	United States of Department Agriculture/ Agricultur	AVP1 - Donor: Arabidopsis thaliana IPT - Donor: Agrobacterium tumefaciens NHX1 - Donor: Arabidopsis thaliana SINA - Donor: Arabidopsis thaliana SINAdn - Donor: Arabidopsis thaliana gus - Donor: Escherichia coli NPTII* - Donor: Escherichia coli A20 -like protein - Donor: Arabidopsis thaliana	AP - Yield Increased MG - Optimization Of Marker Gene Expression
09-103-101N	**Mouse-ear cress**	University of Missouri	Cherry fluorescent tag - Donor: Discosoma sp. PHOT1 protein - Donor: Arabidopsis thaliana bar - Donor: Streptomyces viridochromogenes hpt - Donor: Streptomyces hygroscopicus	AP - Drought Tolerant AP - Hyperphototropic HT - Basta Tolerant MG - Green Fluorescent Protein (Mgfp) And Red Fluoresce

				mGFP - Donor: Aequorea victoria truncated PHOT1 - Donor: Arabidopsis thaliana	
09-093-118N	**Cotton**	United States Department of Agriculture/ Agricultur		En-I - Donor: Zea mays P450 - Donor: Streptomyces griseolus NPTII* - Donor: Escherichia coli bar* - Donor: Streptomyces hygroscopicus	AP - Yield Increased
09-093-113N	**Cotton**	United States Department of Agriculture/ Agricultur		germin-like 1 - Donor: Gossypium hirsutum gus - Donor: Escherichia coli heat shock binding factor 1 - Donor: Arabidopsis thaliana neomycin phosphotransferase II* - Donor: E. coli HPTII* - Donor: Escherichia coli NPTII* - Donor: Escherichia coli SINA - Donor: Arabidopsis thaliana APX - Donor: Pisum sativum IPT - Donor: Agrobacterium tumefaciens GR - Donor: Arabidopsis thaliana GFP - Donor: Aequorea victoria AVP1 - Donor: Arabidopsis thaliana A20 -like protein - Donor: Arabidopsis thaliana NHX1 - Donor: Arabidopsis thaliana BRI-1 - Donor: Arabidopsis thaliana	AP - Yield Increased AP - Drought Tolerance Increased
09-089-112N	**Tobacco**	United States Department of Agriculture/ Agricultur		SBPase Cdna sequence - Donor: Arabidopsis thaliana	AP - Photosynthesis Enhanced
09-080-101N	**Arabidopsis thaliana**	Michigan State University		NPT (neomycin phosphotransferase) from bacterial t* - Donor: NPT (neomycin phosphotransferase) from bacterial t NPT (neomycin phosphotransferase) from bacterial* - Donor: NPT (neomycin phosphotransferase) from bacterial NPT (neomycin phosphotransferase) from bacterial N* - Donor: NPT (neomycin phosphotransferase) from bacterial N M6PR - Donor: Celery Gene: SOS1 from Arabidopsis - Donor: Arabidopsis thaliana CBF3 - Donor: A. thaliana	AP - Dehydration Stress Resistance
09-064-106N	**Potato**	Oregon State University		CBF1 - Donor: Arabidopsis thaliana NPTII* - Donor: bacterial transposon Tn5	AP - Cold Tolerance Increased
09-064-105N	**Soybean**	University of Nebraska/Lincoln		fructose/sedoheptulose bisphosphatase - Donor: Synechoccus sp. transit peptide targets gene to plastid - Donor: P. sativum transit peptide targets protein to plastid - Donor: Pisum sativum	AP - Photosynthesis Enhanced

			selectabel marker, used for transformation* - Donor: Streptomyces hygroscopicus selectable marker gene used for transformation* - Donor: Streptomyces hygroscopicus ICTB, carbon concentrating protein - Donor: Synechoccus elongatus	
09-064-110N	**Potato**	Michigan State University	1577 bp homogentisate phytltransferase (HPT) - Donor: Arabidopsis thaliana 1737 bp hydroxy phenylpyruvate dioxygenase (HPPD) - Donor: Arabidopsis thaliana 900 bp (CBF1) transcription factor - Donor: A. thaliana Bt-Cry1Ia1 - Donor: Bacillus thuringiensis var kurstaki Bt-Cry1a(c) improved - Donor: Bacillus thuringiensis Bt-Cry3A - Donor: Bacillus thuringiensis var tenebrionis RB gene - Donor: Solanum bulbocastanum potato proteinase inhibitor I with avidin - Donor: Gallus gallus neomycin phosphotransferase II (NPT II), Kanamycin* - Donor: Escherichia coli	AP - Cold/Drought Resistance FR - Late Blight Resistant IR - Colorado Potato Beetle Resistant IR - Potato Tuberworm Resistant PQ - Enhanced Nutrition Via Increased Vitamin E
09-064-104N	**Rice**	Arcadia Biosciences	GUS - Donor: Staphylococcus sp gusA hph* - Donor: Streptomyces hygroscopicus AtNHX1 - Donor: Arabidopsis thaliana AlaAT - Donor: Hordeum vulgare	AP - Nitrogen Utilization Efficiency Increase AP - Salt Tolerance Increased MG - Expression Optimization
09-058-109N	**Corn**	University of Illinois	Glossy15 - Donor: Zea mays nptII* - Donor: Escherichia coli Red fluorescent protein - Donor: Discosoma sp. GUS - Donor: Escherichia coli FLOURY2 - Donor: Zea mays PAT* - Donor: Streptomyces hygroscopicus	MG - Seed Color Altered MG - Visual Marker OO - Delayed Shoot Maturation
09-047-112N	**Rapeseed**	Arcadia Biosciences	AlaAT - Donor: Hordeum vulgare CBI* - Donor: CBI NPTII* - Donor: Escherichia coli	AP - Nitrogen Utilization Efficiency Increase
09-030-126N	**Spring wheat**	United States Department of Agriculture/ Agricultur	chloroplast EF-Tu - Donor: Zea mays Neomycin phosphotransferase II (Npt II)* - Donor: E. coli	AP - Heat Tolerance
08-273-101R	**Creeping bentgrass**	Clemson University	Phosphinothricin acetyltransferase (bar) gene - Donor: Streptomyces hygroscopicus Proton-pyrophosphatase gene - Donor: A. thaliana	AP - Drought Tolerance Increased AP - Salt Tolerance Increased HT - Phosphinothricin Tolerant
08-250-101R	**Persimmon**	University of California/Davis	APH(3)II* - Donor: Escherichia coli APH3 II* - Donor: Escherichia coli S6PDH - Donor: Malus domestica	AP - Drought Tolerance Increased IR - Lepidopteran Insect Resistant MG - Visual Marker

			beta glucuronidase - Donor: Escherichia coli codA - Donor: Arthrobacter globiformis cryIAc - Donor: Bacillus thuringiensis uidA - Donor: Escherichia coli	
08-235-102R	**Apple**	University of California/Davis	ACC oxidase - Donor: Malus domestica ACC synthase - Donor: Apple S6PDH Sorbitol 6 phosphate dehydrogenase - Donor: Apple beta glucuronidase - Donor: E.coli beta glucuronidase - Donor: Escherichia coli nptII* - Donor: E.coli Transposable element Tn5 nptII* - Donor: E.coli transposable element Tn5	PQ - Ethylene Supression PQ - Sorbitol Levels Altered
08-142-108N	**Corn**	Cold Spring Harbor Laboratory	TONOPLAST INTRINSIC PROTEIN1 (TIP1) - Donor: Zea mays Red fluorescent protein - Donor: Discosoma sp. RESPONSE REGULATOR 7(RR7) - Donor: Zea mays RESPONSE REGULATOR 1(RR1) - Donor: Zea mays Transcription factor LhG4 - Donor: S. cerevisiae RAMOSA1 - Donor: Zea mays red fluorescent protein - Donor: Discosoma sp. PINFORMED1 - Donor: Zea mays RAMOSA3 - Donor: Zea mays Yellow fluorescent protein (YFP) - Donor: A. victoria Yellow fluorescent protein - Donor: Aequorea victoria cyan fluorescent protein - Donor: Aequorea victoria trehalase gene, treF - Donor: Escherichia coli yellow fluorescent protein - Donor: Aequorea victoria phosphinothricin acetyltransferase* - Donor: Streptomyces hygroscopicus Fluorescent protein fusion - Donor: Zea mays and Aequorea victoria PHYTOENE SYNTHASE - Donor: Zea mays b-glucuronidase, GUS - Donor: Escherichia coli FASCIATED EAR2 - Donor: Zea mays Alpha Tubulin - Donor: Zea mays CYTOKININ OXIDASE 1 - Donor: Zea mays CYTOKININ OXIDASE 2 - Donor: Zea mays PEROXIN11 - Donor: Zea mays FASCIATED EAR2 gene-epitope tagged - Donor: Zea mays FASCIATED EAR2- epitope tagged - Donor: Zea mays	MG - Visual Marker OO - Epitope Tagged Gene AP - Seed Number Increased AP - Inflorescence Development Altered AP - Floral Development Altered AP - Branching Decreased OO - Transactivated Expression

			FLOURY2 - Donor: Zea mays LhG4 synthetic transactivator - Donor: S. cerevisiae Cyclin D2 gene - Donor: Zea mays Mutator B gene - Donor: Zea mays HISTIDINE KINASE2 (HK2) - Donor: Zea mays ISOPENTENYL TRANSFERASE 2 (IPT2) - Donor: Zea mays Histone H1 - Donor: Zea mays Histidine phosphotransferase - Donor: Zea mays ABPHYL1 - Donor: Zea mays Glucocorticoid receptor - Donor: Rattus norvegicus	
08-109-101N	**Cotton**	United States Department of Agriculture/ Agricultur	NPTII selectable marker* - Donor: Escherichia coli APX - Donor: Pisum sativum	AP - Drought Tolerance Increased
08-105-105N	**Potato**	Michigan State University	900 bp (CBF1) transcription factor - Donor: Arabidopsis neomycin phosphotransferase II (NPT II)* - Donor: Escherichia coli	OO - Cold/Drought Resistance Gene
08-105-103N	**Potato**	Michigan State University	900 bp (CBF1) transcription factor - Donor: Arabidopsis neomycin phosphotransferase II (NPT II)* - Donor: Escherichia coli	OO - Cold/Drought Resistance Gene
08-089-101N	**Corn**	University of Illinois	FLOURY2 - Donor: Zea mays nptII* - Donor: Escherichia coli PAT* - Donor: Streptomyces hygroscopicus neomycin phosphotransferase* - Donor: Escherichia coli Red fluorescent protein - Donor: Discosoma sp. GUS - Donor: Escherichia coli Glossy15 - Donor: Zea mays	OO - Prolonged Juvenile Vegetative Phase MG - Visual Marker MG - Seed Color Altered
08-088-104N	**Tobacco**	University of California/Davis	IPT-isopentenyltransferase-synthesis of cytokinin - Donor: Agrobacterium tumefaciens antibiotic resistance - Donor: Escherichia coli	AP - Drought Tolerance Increased
08-087-107N	**Plum**	United States Department of Agriculture/ Agricultur	NPT II* - Donor: Escherichia coli Flowering Time 1 - Donor: Populus trichocarpa	AP - Flowering Time Altered MG - Kanamycin Resistant
08-086-104N	**Rice**	Arcadia Biosciences	AlaAT - Donor: Hordeum vulgare AtNHX1 - Donor: Arabidopsis thaliana GUS - Donor: Staphylococcus sp gusA hph* - Donor: Streptomyces hygroscopicus	AP - Nitrogen Utilization Efficiency Increase MG - Expression Optimization AP - Salt Tolerance Increased
08-081-103N	**Soybean**	University of Missouri/Delta Center	AtDREB1D - Donor: Arabidopsis thaliana Bar - Donor: Streptomyces Hygroscopicus	AP - Drought Tolerance
08-077-104R	**Hybrid poplar**	Purdue University	Cytochrome P450 - Donor: Oryctolagus cuniculus	OO - Control

			NPTII* - Donor: Tn5 transposon from E. coli	OO - Phytoremediation
08-073-110N	**Cotton**	United States of Department Agriculture/ Agricultur	AVP1 - Donor: Arabidopsis thaliana BRI-1 anti-sense - Donor: Arabidopsis thaliana BRI-1 - Donor: Arabidopsis thaliana germin-like protein 1 - Donor: Gossypium hirsutum glutathione reductase - Donor: Arabidopsis thaliana heat shock binding factor 1 - Donor: Arabidopsis thaliana NPT II* - Donor: Escherichia coli NPTII* - Donor: Escherichia coli A20 like protein - Donor: Arabidopsis thaliana	AP - Drought Tolerance Increased AP - High Temperature Tolerant AP - Yield Increased
08-067-110N	**Tomato**	Purdue University	AVP1 proton pyrophosphatase - Donor: Arabidopsis thaliana neomycin phosphotransferase* - Donor: Escherichia coli	PQ - Enhanced Drought Resistance And Phosphorous Uptake
08-065-112N	**Poplar**	Mississippi State University	CONSTANS2 - Donor: Populus deltoides aminoglycoside 3 -phosphotransferase* - Donor: Corynebacterium diptheriae aminoglycoside 3 phosphotransferase* - Donor: Corynebacterium diptheriae PHYTOCHROME B - Donor: Populus deltoides PHYTOCHROME A - Donor: Populus deltoides FLOWERING LOCUS T2 - Donor: Populus deltoides CONSTANS1 - Donor: Populus deltoides APETALA1 - Donor: Populus deltoides GIBBERELLIN INSENSITIVE - Donor: Populus deltoides	AP - Development Altered
08-065-104N	**Arabidopsis thaliana**	Michigan State University	CBF3 (C-repeat binding factor) - Donor: Arabidopsis thaliana M6PR - Donor: celery SOS1 (salt sensitive 1) - Donor: Arabidopsis thaliana	AP - Dehydration Stress Resistance AP - Dehydratoin Stress Resistance
08-056-103R	**Guayule**	United States of Department Agriculture/ Agricultur	AT-Hook - Donor: Arabidopsis thaliana G350 - Donor: Arabidopsis thaliana G47 - Donor: Arabidopsis thaliana Npt II* - Donor: Escherichia coli Npt II - Donor: Escherichia coli none empty vector - Donor: None	AP - Drought Tolerance Increased AP - Yield Increased OO - Vector Control PQ - Rubber Yield Increased
08-029-107N	**Rice**	Arcadia Biosciences	AtNHX1 - Donor: Arabidopsis thaliana GUS - Donor: Staphylococcus sp gusA hph* - Donor: Streptomyces hygroscopicus AlaAT - Donor: Hordeum vulgare	AP - Nitrogen Utilization Efficiency Increase MG - Expression Optimization AP - Salt Tolerance Increased
07-315-101R	**Poplar**	Mississippi State University	ACTIVATING SIGNAL COINTEGRATOR 1 - Donor: Populus deltoides	AP - Floral Development Altered

				APETALA1 - Donor: Populus deltoides CONSTANS1 - Donor: Populus deltoides CONSTANS2 - Donor: Populus deltoides FLOWERING LOCUS T2 - Donor: Populus deltoides GIBBERELLIN INSENSITIVE - Donor: Populus deltoides PHYTOCHROME A - Donor: Populus deltoides PHYTOCHROME B - Donor: Populus deltoides aminoglycoside 3 phosphotransferase* - Donor: Corynebacterium diptheriae aminoglycoside 3 -phosphotransferase* - Donor: Corynebacterium diptheriae	AP - Flowering Time Altered
07-268-102N	**Poplar**	Oregon University	State	PTLF and PTAG - Donor: Populus trichocarpa PTAP - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa PTAG - Donor: Populus trichocarpa beta-glucuronidase (GUS) - Donor: Escherichia coli AGAMOUS (AG) - Donor: Arabidopsis thaliana NPTII* - Donor: Escherichia coli BARNASE - Donor: Bacillus amyloliquifaciens PSVP - Donor: Populus trichocarpa APETALA 1 (AP1) - Donor: Arabidopsis thaliana BARSTAR - Donor: Bacillus amyloliquifaciens PAGL20 (MADS 5) - Donor: Populus trichocarpa PCEN-L - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PFT - Donor: Populus trichocarpa PMFT - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa	OO - Reproductive Fertility Altered MG - Visual Marker OO - Flowering Fertility Altered OO - Flowering Time Altered
07-268-101N	**Poplar**	Oregon University	State	PTAP - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli beta-glucuronidase (GUS) - Donor: Eschericia coli PTLF, PTAP1, and PTAG genes - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa	MG - Visual Marker OO - Flowering Time Altered OO - Reproductive Fertility Altered

				PTD - Donor: Populus trichocarpa PTAP1 and PTAG1 genes - Donor: Populus trichocarpa PTAG - Donor: Populus trichocarpa PSVP - Donor: Populus trichocarpa BARSTAR - Donor: Bacillus amyloliquifaciens PTAP1 and PTLF - Donor: Populus trichocarpa PFT - Donor: Populus trichocarpa BARNASE - Donor: Bacillus amyloliquifaciens AGAMOUS (AG) - Donor: Arabidopsis thaliana Inverted repeat of PAGL20 (MADS 5) - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PCEN-L - Donor: Populus trichocarpa PFPFL1 - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PFT and PAGL20 genes - Donor: Populus trichocarpa APETALA 1 (AP1) - Donor: Arabidopsis thaliana	
07-268-103N	**Poplar**	Oregon University	State	NPT11* - Donor: Eschericia coli PTAG - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli beta-glucuronidase (GUS) - Donor: Eschericia coli PSVP - Donor: Populus trichocarpa PTAP - Donor: Populus trichocarpa PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa PFT and PAGL20 - Donor: Populus trichocarpa AGAMOUS (AG) - Donor: Arabidopsis thaliana PFT and PAGL20 genes - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PFPFL1 - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PAGL20(MADS 5) - Donor: Populus trichocarpa BARSTAR - Donor: Bacillus amyloliquifaciens BARNASE - Donor: Bacillus amyloliquifaciens	OO - Reproductive Fertility Altered OO - Flowering Time Altered MG - Visual Marker

			APETALA 1 (AP1) - Donor: Arabidopsis thaliana PFT - Donor: Populus trichocarpa	
07-253-101N	**Rapeseed**	Arcadia Biosciences	AlaAT - Donor: Hordeum vulgare CBI* - Donor: CBI NPTII* - Donor: Escherichia coli	AP - Nitrogen Utilization Efficiency Increase
07-234-102N	**Oilseed rape**	University of Tennessee	gibberellic acid insensitive dwarfing gene - Donor: Arabidopsis thaliana nptII - Donor: Escherichia coli mGFP5-ER - Donor: Aequorea, Arabidopsis GFP - Donor: Aequorea victoria Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki nptII* - Donor: Escherichia coli acetohydroxyacid synthase (ahas) - Donor: A. thaliana	IR - Corn Earworm Resistant HT - Sulfonylurea Tolerant MG - Visual Marker AP - Dwarfed
07-234-101N	**Oilseed rape/field mustard x oilseed rape hybrids**	University of Tennessee	Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki GFP - Donor: Aequorea victoria acetohydroxyacid synthase (ahas) - Donor: A. thaliana gibberellic acid insensitive dwarfing gene - Donor: Arabidopsis thaliana mGFP5-ER - Donor: Aequorea, Arabidopsis nptii - Donor: Escherichia coli nptii* - Donor: Escherichia colo	MG - Visual Marker AP - Dwarfed HT - Sulfonylurea Tolerant IR - Corn Earworm Resistant
07-234-103N	**Oilseed rape/field mustard**	University of Tennessee	nptii* - Donor: Escherichia colo Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki GFP - Donor: Aequorea victoria acetohydroxyacid synthase (ahas) - Donor: A. thaliana gibberellic acid insensitive dwarfing gene - Donor: Arabidopsis thaliana mGFP5-ER - Donor: Aequorea, Arabidopsis nptii - Donor: Escherichia coli	IR - Corn Earworm Resistant MG - Visual Marker AP - Dwarfed HT - Sulfonylurea Tolerant
07-232-102R	**Sweetgum**	Oregon State University	AG-M3 - Donor: Arabidopsis thaliana BARNASE - Donor: Bacillus amyloliquefaciens BARSTAR - Donor: Bacillus amyloliquifaciens LSAG/ LAG - Donor: Liquidambar styraciflua NPT II* - Donor: Escherichia coli	OO - Modified Flowering
07-197-127R	**Bahiagrass**	University of Florida	C-repeat binding factor - Donor: Hordeum sponttaneum Gibberellin 2-oxidasel - Donor: Arabidopsis thaliana Homeobox16 - Donor: Arabidopsis thaliana PhytochromeA - Donor: oat	AP - Increased Cold Tolerance AP - Increased Drought Tolerance AP - Plant Height Reduced

		TALE-Homeobox16 - Donor: Arabidopsis thaliana dehydran - Donor: Hordeum spontaneum drought inducible transcription factor - Donor: barley drought inducible transcription factor - Donor: rice	
07-142-102N	**Arabidopsis thaliana**	Michigan State University	Mannose 6 phosphate reductase - Donor: Apium graveolens Na/H antiporter - Donor: Arabidopsis thaliana NPT* - Donor: Escherichia coli npt* - Donor: Escherichia coli C-repeat binding factor 3 - Donor: Arabidopsis thaliana	AP - Dehydration Stress Resistance
07-137-102N	**Cotton**	United States Department of Agriculture/ Agricultur	AVP1 - Donor: Arabidopsis neomycin phosphotransferase II* - Donor: Escherichia coli	AP - Drought Stress Tolerance
07-128-101R	**Poplar**	University of Connecticut	DET2 - Donor: Cotton FLP - Donor: Yeast GA20 - Donor: Cotton GUS* - Donor: Escherichia coli kanamycin resistance* - Donor: Staphylococcus aureus nptII* - Donor: Staphylococcus aureus	AP - Flowering Altered AP - Growth Promotion Or No Visible Change In Phenotype
07-116-101N	**Rapeseed**	Arcadia Biosciences	AlaAT - Donor: Hordeum vulgare CBI* - Donor: CBI NPTII* - Donor: Escherichia coli	AP - Nitrogen Utilization Efficiency Increase
07-115-101N	**Tomato**	Purdue University	pectin methylesterase (PME) - Donor: Lyc. esculentum neomycin phosphotransferase* - Donor: Escherichia coli neomycin phosphotransferase (npt)* - Donor: E. coli Antisense Vis1 a sHSP (Acce. AY128102) - Donor: Lycopersicon esculentum Vis1 a sHSP (Acce. AY128102) - Donor: Lyc. esculentum Spermidine synthase - Donor: Saccharomyces cerevisiae Polygalacturonase 2 - Donor: Lycopersicon esculentum Spermidine synthase (SPE3) - Donor: S. cerevisiae	PQ - Polygalacturonase Level Reduced PQ - Heat Stress Tolerance PQ - Fruit Ripening Altered PQ - Fruit Pectin Esterase Level Decreased
07-110-102N	**Cotton**	Texas Tech University	GR - Donor: Arabidopsis thaliana NPT II* - Donor: Escherichia coli APX - Donor: Pisum sativum AOX1 - Donor: Nicotiana tabacum CAT - Donor: Zea mays	AP - Oxidative Stress Tolerant AP - Cold Tolerance Increased
07-110-101N	**Rice**	Arcadia Biosciences	hph* - Donor: Streptomyces hygroscopicus OsNHX1+UTRs - Donor: Oryza sativa OsNHX1 - Donor: Oryza sativa	AP - Salt Tolerance Increased AP - Nitrogen Utilization Efficiency

			GUS - Donor: Escherichia coli AtNHX1+UTRs - Donor: Arabidopsis thaliana AlaAT - Donor: Hordeum vulgare AtNHX1 - Donor: Arabidopsis thaliana	Increase
07-093-104N	**Cotton**	Texas Tech University	neomycin phosophotransferase II* - Donor: E. coli AVP1 - Donor: Arabidopsis vacuolar proton-ATPase	AP - Drought Stress Tolerance
07-090-101N	**Corn**	University of Illinois	neomycin phosphotransferase* - Donor: Escherichia coli GUS - Donor: Escherichia coli Glossy15 - Donor: Zea mays	OO - Prolonged Juvenile Vegetative Phase MG - Visual Marker
07-086-105N	**Poplar**	Oregon State University	APETALA2 - Donor: Arabidopsis thaliana STERILE APETALA - Donor: Arabidopsis thaliana NPT II* - Donor: Escherichia coli	OO - Curly Leaves OO - Bigger Leaves
07-082-104N	**Cotton**	United States Department of Agriculture/ Agricultur	Athsp101 - Donor: Arabidopsis thaliana NPT II* - Donor: Escherichia coli	AP - High Temperature Tolerant
07-081-116N	**Potato**	Michigan State University	CBF1 transcription factor - Donor: Arabidopsis neomycin phosphotransferase II (NPT II), Kanamycin* - Donor: Escherichia coli	OO - Cold Resistance OO - Drought Resistance
07-081-117N	**Potato**	Michigan State University	neomycin phosphotransferase II (NPT II), Kanamycin* - Donor: Escherichia coli CBF1 transcription factor - Donor: Arabidopsis	OO - Cold Resistance OO - Drought Resistance
07-080-104N	**Poplar**	Oregon State University	GAI - Donor: Arabidopsis thaliana PcGA2ox1 - Donor: Phaseolus coccineus PtaGA2ox1 - Donor: Populus tremula x P. alba RGL1 - Donor: Arabidopsis thaliana NPTI* - Donor: Corynebacterium diphtheriae NPTII* - Donor: Escherichia coli	OO - Reduced Stature
07-080-106N	**Corn**	University of Wisconsin/ Madison	Phosphinothricin acetyltransferase (bar) - Donor: Streptomyces hygroscopicus TGA1, ZFL2, ZAGL1 , TB1, TGA1 - Donor: maize	PQ - Altered Flowering
07-078-101N	**Tomato**	University of California/Davis	NPTII* - Donor: Escherichia coli Phosphinothricin acetyltransferase* - Donor: Streptomyces hygroscopicus Meristem expression - Donor: Arabidopsis thaliana AP2 Transcription Factor - Donor: Arabidopsis thaliana	AP - Yield Increased
07-071-103N	**Rice**	Arcadia Biosciences	AtNHX1 - Donor: Arabidopsis thaliana AtNHX1+UTRs - Donor: Arabidopsis thaliana GUS - Donor: Escherichia coli	AP - Salt Tolerance Increased AP - Improved Nitrogen Utilization

			OsNHX1 - Donor: Oryza sativa OsNHX1+UTRs - Donor: Oryza sativa hph* - Donor: Streptomyces hygroscopicus AlaAT - Donor: Hordeum vulgare	
07-069-101N	**Poplar**	Mississippi State University	APETALA1 - Donor: Populus deltoides CONSTANS1 - Donor: Populus deltoides CONSTANS2 - Donor: Populus deltoides FLOWERING LOCUS T2 - Donor: Populus deltoides GIBBERELLIN INSENSITIVE - Donor: Populus deltoides PHYTOCHROME A - Donor: Populus deltoides PHYTOCHROME B - Donor: Populus deltoides aminoglycoside 3 phosphotransferase* - Donor: Corynebacterium diptheriae aminoglycoside 3 -phosphotransferase* - Donor: Corynebacterium diptheriae	AP - Development Altered
07-065-125N	**Corn**	Cold Spring Harbor Laboratory	b-glucuronidase, GUS - Donor: Escherichia coli RAMOSA1 - Donor: Zea mays RESPONSE REGULATOR 1(RR1) - Donor: Zea mays RESPONSE REGULATOR 7(RR7) - Donor: Zea mays Red fluorescent protein - Donor: Discosoma sp. TONOPLAST INTRINSIC PROTEIN1 (TIP1) - Donor: Zea mays Transcription factor LhG4 - Donor: S. cerevisiae PHYTOENE SYNTHASE - Donor: Zea mays Yellow fluorescent protein - Donor: Aequorea victoria PINFORMED1 - Donor: Zea mays cyan fluorescent protein - Donor: Aequorea victoria trehalase gene, treF - Donor: Escherichia coli yellow fluorescent protein - Donor: Aequorea victoria phosphinothricin acetyltransferase* - Donor: Streptomyces hygroscopicus Yellow fluorescent protein (YFP) - Donor: A.victoria CYTOKININ OXIDASE 1 - Donor: Zea mays RAMOSA3 - Donor: Zea mays Alpha Tubulin - Donor: Zea mays PEROXIN11 - Donor: Zea mays CYTOKININ OXIDASE 2 - Donor: Zea mays FASCIATED EAR2 gene-epitope tagged - Donor: Z. mays FASCIATED EAR2 - Donor: Zea mays	AP - Floral Development Altered OO - Transactivated Expression OO - Epitope Tagged Gene AP - Inflorescence Development Altered AP - Seed Number Increased MG - Visual Marker

			FASCIATED EAR2- epitope tagged - Donor: Zea mays Fluorescent protein fusion - Donor: Zea mays and Aequorea victoria Glucocorticoid receptor - Donor: Rattus norvegicus HISTIDINE KINASE2 (HK2) - Donor: Zea mays Histone H1 - Donor: Zea mays ISOPENTENYL TRANSFERASE 2 (IPT2) - Donor: Zea mays LhG4 synthetic transactivator - Donor: S. cerevisiae Mutator B gene - Donor: Zea mays ABPHYL1 - Donor: Zea mays FLOURY2 - Donor: Zea mays	
07-029-102N	**Wheat**	Oklahoma State University	mannitol-1-phosphate dehydrogenase - Donor: E. coli phosphinothricin acetyltransferase (bar)* - Donor: Streptomyces hygroscopicus	HT - Selectable Marker/Herbicide Tolerant AP - Drought Tolerance
06-324-108N	**Corn**	Cold Spring Harbor Laboratory	phosphinothricin acetyltransferase (bar)* - Donor: Streptomyces hygroscopicus Ramosa3 gene - Donor: Zea mays	AP - Branching Increased
06-319-103N	**Cotton**	Arcadia Biosciences	AtNHX1 - Donor: Arabidopsis thaliana NPTII* - Donor: Escherichia coli	AP - Salinity Tolerance
06-305-01R	**Creeping bentgrass**	Rutgers University	Hygromycin resistance & GUS* - Donor: Escherichia coli isopentenyl transferase - Donor: Agrobac. tumefaciens	AP - Heat Tolerance
06-283-101N	**Poplar**	Oregon State University	PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli PTAP - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa beta-glucuronidase (GUS) - Donor: Eschericia coli PMFT - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa BARNASE - Donor: Bacillus amyloliquifaciens PTAG - Donor: Populus trichocarpa PSVP - Donor: Populus trichocarpa APETALA 1 (AP1) - Donor: Arabidopsis thaliana BARSTAR - Donor: Bacillus amyloliquifaciens PAGL20 (MADS 5) - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa	OO - Flowering Fertility Altered OO - Flowering Time Altered OO - Reproductive Fertility Altered MG - Visual Marker

				PCEN-L - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PFT - Donor: Populus trichocarpa AGAMOUS (AG) - Donor: Arabidopsis thaliana	
06-282-103N	**Poplar**	Oregon University	State	PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa PTAG - Donor: Populus trichocarpa PTAP - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa NPT11* - Donor: Eschericia coli PTLF - Donor: Populus trichocarpa beta-glucuronidase (GUS) - Donor: Eschericia coli PSVP - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli PTLF and PTAG - Donor: Populus trichocarpa BARSTAR - Donor: Bacillus amyloliquifaciens PTD - Donor: Populus trichocarpa AGAMOUS (AG) - Donor: Arabidopsis thaliana PFT - Donor: Populus trichocarpa BARNASE - Donor: Bacillus amyloliquifaciens PAGL20(MADS 5) - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PFPFL1 - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PFT and PAGL20 genes - Donor: Populus trichocarpa PFT and PAGL20 - Donor: Populus trichocarpa APETALA 1 (AP1) - Donor: Arabidopsis thaliana	OO - Flowering Time Altered MG - Visual Marker OO - Reproductive Fertility Altered
06-282-102N	**Poplar**	Oregon University	State	PSVP - Donor: Populus trichocarpa beta-glucuronidase (GUS) - Donor: Eschericia coli PTLF, PTAP1, and PTAG genes - Donor: P. trichocarpa PTLF - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa PTAP1 and PTAG1 genes - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PTAP - Donor: Populus trichocarpa PTAG - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli	OO - Reproductive Fertility Altered OO - Flowering Time Altered MG - Visual Marker

				APETALA 1 (AP1) - Donor: Arabidopsis thaliana PFT - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa AGAMOUS (AG) - Donor: Arabidopsis thaliana BARNASE - Donor: Bacillus amyloliquifaciens BARSTAR - Donor: Bacillus amyloliquifaciens Inverted repeat of PAGL20 (MADS 5) - Donor: Populus trichocarpa PCEN-L - Donor: Populus trichocarpa PFPFL1 - Donor: Populus trichocarpa PFT and PAGL20 genes - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa	
06-272-103N	**Cotton**	United States of Department Agriculture/ Agricultur		glutathione reductase - Donor: Arabidopsis thaliana manganese superoxide dismutase - Donor: Nicotiana plumbaginifolia catalase 3 - Donor: Zea mays Heat Shock Binding Factor 1 - Donor: A. thaliana Germin-like protein - Donor: Gossypium hirsutum An1-like zinc finger protein - Donor: Arabidopsis thaliana ascorbate peroxidase - Donor: Pisum sativum neomycin phosphotransferase II* - Donor: E. coli	AP - Stress Tolerance
06-270-113N	**Poplar**	Oregon State University		PMFT - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli beta-glucuronidase (GUS) - Donor: Eschericia coli PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa PTAP - Donor: Populus trichocarpa PSVP - Donor: Populus trichocarpa PFT - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa PCEN-L - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PAGL20 (MADS 5) - Donor: Populus trichocarpa BARSTAR - Donor: Bacillus amyloliquifaciens	MG - Visual Marker OO - Flowering Fertility Altered OO - Reproductive Fertility Altered OO - Flowering Time Altered

				BARNASE - Donor: Bacillus amyloliquifaciens APETALA 1 (AP1) - Donor: Arabidopsis thaliana AGAMOUS (AG) - Donor: Arabidopsis thaliana PTAG - Donor: Populus trichocarpa	
06-263-107N	**Poplar**	Oregon University	State	PTAP - Donor: Populus trichocarpa NPTII* - Donor: Eschericia coli NPT11* - Donor: Eschericia coli beta-glucuronidase (GUS) - Donor: Eschericia coli PTLF, PTAP1, and PTAG - Donor: Populus trichocarpa PTLF - Donor: Populus trichocarpa PTLF and PTAG - Donor: Populus trichocarpa PTD - Donor: Populus trichocarpa PTAP1 and PTLF - Donor: Populus trichocarpa PTAP1 and PTAG1 - Donor: Populus trichocarpa PFPFL2 - Donor: Populus trichocarpa AGAMOUS (AG) - Donor: Arabidopsis thaliana PSVP - Donor: Populus trichocarpa PFT and PAGL20 - Donor: Populus trichocarpa PFT - Donor: Populus trichocarpa PFPFL1 - Donor: Populus trichocarpa PAGL24 (MADS 9) - Donor: Populus trichocarpa PAGL20(MADS 5) - Donor: Populus trichocarpa BARSTAR - Donor: Bacillus amyloliquifaciens BARNASE - Donor: Bacillus amyloliquifaciens APETALA 1 (AP1) - Donor: Arabidopsis thaliana PTAG - Donor: Populus trichocarpa	MG - Visual Marker OO - Reproductive Fertility Altered OO - Flowering Time Altered
06-254-102N	**Rapeseed**	Arcadia Biosciences		alanine aminotransferase - Donor: barley alanine amonitransferase - Donor: barley CBI* - Donor: CBI NPT II* - Donor: Escherichia coli NPTII* - Donor: Escherichia coli	AP - Nitrogen Utilization Efficiency Increase
06-250-01R	**Poplar**	Oregon University	State	4CL - Donor: Populus tremuloides AG - Donor: Arabidopsis thaliana AP1 - Donor: Arabidopsis thaliana AtSPY - Donor: Arabidopsis thaliana BAR* - Donor: Streptomyces hygroscopicus Bar* - Donor: Streptomyces hygroscopicus	IR - Coleopteran Resistant MG - Visual Marker OO - Altered Gene Expression OO - Altered Growth OO - Empty Transformation Vector OO - Enhanced Growth

			Barnase - Donor: Bacillus amyloliquefaciens	OO - Gibberellin Altered
			Barstar - Donor: Bacillus amyloliquefaciens	OO - Reduced Stature
			Cry3a - Donor: Bacillus thuringiensis	OO - Reduces Stature
			DTA - Donor: Corynebacterium diptheriae	OO - Reproductive Fertility Altered
			GA20ox7 - Donor: Populus trichocarpa	PQ - Wood Quality Altered
			GAI - Donor: Arabidopsis thaliana	
			Gfp* - Donor: Aequorea victoria	
			HvSPY - Donor: Hordeum vulgare	
			NPT II* - Donor: Escherichia coli	
			None - Donor: None	
			NptII* - Donor: Escherichia coli	
			PAGL24 - Donor: Populus trichocarpa	
			PCENL - Donor: Populus trichocarpa	
			PFCL - Donor: Populus trichocarpa	
			PHYB1 - Donor: Arabidopsis thaliana	
			PHYB2 - Donor: Arabidopsis thaliana	
			PMFT - Donor: Populus trichocarpa	
			PSVP - Donor: Populus trichocarpa	
			PTAG - Donor: Populus trichocarpa	
			PTAP - Donor: Populus trichocarpa	
			PTD - Donor: Populus trichocarpa	
			PTLF - Donor: Populus trichocarpa	
			PcGA2 OXI1 - Donor: Phaseolus coccineus	
			PcGA2ox1 - Donor: Phaseolus coccineus	
			PtaGA2 OXI1 - Donor: Populus tremula x alba	
			PtaGA2ox1 - Donor: Populus tremula x P. alba	
			RGL-1 - Donor: Arabidopsis thaliana	
			RGL1 - Donor: Arabidopsis thaliana	
			Transcription factor - Donor: Populus tremula x alba	
			Transcription regulator - Donor: Populus tremula x alba	
			none - Donor: none	
06-242-03R	**Nicotiana attenuata**	Max Planck Institute for Chemical Ecology	1,2-oxo-phytodienic-acid reductase - Donor: N. attenuata	MG - Similar To Wildtype
			1-aminocyclopropane-1-carboxylic acid oxidase - Donor: Nicotiana attenuata	NR - Ethylene Insensitive
			3-Hydroxy-3-methylglutaryl-CoA-reductase - Donor: Nicotiana attenuata	OO - Altered Growth Pattern, Susceptible To Herbivory
			35s - Donor: Cauliflower mosaic caulimovirus	OO - Altered Responses To Light
			Allene Oxide Cyclase - Donor: Nicotiana attenuata	OO - Altered Volatile Composition

Allene oxide synthase - Donor: Nicotiana attenuata	OO - Changed Leaf Volatile Composition
Alpha-dioxygenase - Donor: Nicotiana attenuata	OO - Constitutive Expression Of Coi1-Regulated Defenses
Alternative oxidase - Donor: Nicotiana attenuata	OO - Constitutive/Elevated Bergamotene Production
Calcium dependent protein kinase - Donor: Nicotiana attenuata	OO - Decreased Local And Systemic Resistance
Calcium dependent protein kinase 5 - Donor: Nicotiana attenuata	OO - Decreased Phytohormones
Carbohydrate oxidase similar - Donor: Nicotiana attenuata	OO - Decreased Phytohormones And Reduced Phytohormone R
Chalcone synthase - Donor: Nicotiana attenuata	OO - Decreased Phytohormones And Reduced Sensitivity To
Cinnamic acid 4-hydroxylase - Donor: Nicotiana attenuata	OO - Decreased Phytohormones And Reduced Xyloglucan End
Cinnamoyl alcohol dehydrogenase - Donor: Nicotiana attenuata	OO - Decreased Phytohormones, Reduced Nicotine, And Red
Coronatine Insensitive 1 - Donor: Nicotiana attenuata	OO - Delayed Scenescence
Coronatine-Insensitive 1 - Donor: Nicotiana attenuata	OO - Dwarf Plants, High Sa And Aba, Defficient In Ja Bu
Dicer like protein 1 - Donor: Nicotiana attenuata	OO - Empty Transformation Vector
Dicer like protein 4 - Donor: Nicotiana attenuata	OO - Empty Vector Control
Ethylene receptor - Donor: Arabidopsis thaliana	OO - Enhanced Herbivore Resistance And Suseptible To Pa
Ethylene receptor mutant - Donor: Arabidopsis thaliana	OO - Enhanced Herbivory
Ethylene receptor mutant (etr1-1) - Donor: Arabidopsis thaliana	OO - Enhanced Herbivory, Low Content Of Hydroxycinnamoy
F-box protein-like - Donor: Nicotiana attenuata	OO - Ethylene Insensitive
Farnesyl pyrophosphate synthase - Donor: Nicotiana attenuata	OO - Ethylene Insensitive And Reduced Benzylacetone Emi
GFP-Sporamin - Donor: Ipomoea batatas	OO - Ethylene Sensitive, Reduced Volatiles, And Reduced
GFP-Ubiquitin - Donor: Plantago major	OO - Ethylene Sensitive, Reduced Volatiles, Reduced Pis
Gamma-thionin - Donor: Nicotiana attenuata	OO - Ethylene Sensitive,Reduced Volatles, And Reduced P
Gamma-thionine - Donor: Nicotiana attenuata	OO - Growth Altered
Geranylgeranyl pyrophosphate synthase - Donor: Nicotiana attenuata	OO - Herbivore Defense Decreased
Germin - Donor: Nicotiana attenuata	OO - Herbivore Deterrent
Glyceraldehyde-3-phosphate dehydrogenase - Donor: Nicotiana attenuata	OO - Herbivore Resistant
Herbivory induced gene - Donor: Nicotiana attenuata	OO - Higher Herbivore, Pathogen Resistance
Hsp90 isoform 1 - Donor: Nicotiana attenuata	OO - Higher Pathogen Resistance, Less Herbivore Resista
Hsp90 isoform 2 - Donor: Nicotiana attenuata	OO - Increased Disease Resistance
Hydroperoxide lyase - Donor: Arabidopsis thaliana	OO - Increased Herbivore Resistance
Hydroperoxide lyase - Donor: Nicotiana attenuata	OO - Increased Nicotine Levels
Hygromycin phosphotranferase* - Donor: Escherichia coli	OO - Increased Photosynthetic Rates
Hygromycin phosphotransferase* - Donor: Escherichia coli	OO - Increased Resistance To Herbivores, Pathogens And
Lectin - Donor: Nicotiana tabacum	OO - Increased Sucrose Export
Lipid transferase - Donor: Nicotiana attenuata	OO - Increased Systemin Polypeptide
Lipoxygenase - Donor: Nicotiana attenuata	OO - Less Heat, Herbivore, Pathogen Resistance
Lipoxygenase (LOX2) - Donor: Nicotiana attenuata	OO - Less Resistance To Herbivores

Lipoxygenase (LOX3) - Donor: Nicotiana attenuata	OO - Less Resistance To Herbivores And Pathogens
Lipoxygenase gene (LOX3) - Donor: Nicotiana attenuata	OO - Lower Jasmonic Acid Levels, Reduced Herbivore Defe
Lipoxygenase3 - Donor: Nicotiana attenuata	OO - Lower Lignin Content And Structural Rigidity
MAP kinase 4 - Donor: Nicotiana attenuata	OO - More Competitive
MYB Transcription Factor - Donor: Nicotiana attenuata	OO - Photosynthetic Rates Reduced
Methyl Jasmonate esterase - Donor: Nicotiana attenuata	OO - Reduced Alternative Oxidase
Metyl-Jasmonate esterase - Donor: Nicotiana attenuata	OO - Reduced Benzylacetone Emission From Flowers
NAC/NAM transcription factor - Donor: Nicotiana attenuata	OO - Reduced Benzylacetone Emission From Flowers And Re
NADH dehydrogenase subunit - Donor: Nicotiana attenuata	OO - Reduced Benzylacetone Emission From Flowers, Reduc
NADPH oxidase (NarbohD) - Donor: Nicotiana attenuata	OO - Reduced C6 Volatiles And Constitutive/Elevated Ber
NaF4 (Salicylic Acid inducible protein kinase homo - Donor: Nicotiana attenuata	OO - Reduced C6 Volatiles And Reduced Nicotine
Nitrate reductase - Donor: Nicotiana attenuata	OO - Reduced C6 Volatiles And Reduced Proteinase Inhibi
None - Donor: None	OO - Reduced C6 Volatiles, Reduced Nicotine, And Reduce
PR1- (pathogen resistance protein 1) - Donor: Nicotiana attenuata	OO - Reduced Calcium Dependent Protein Kinase
Pathogen resistance protein 1 - Donor: Nicotiana attenuata	OO - Reduced Ethylene
Pectin methyl esterase - Donor: Nicotiana attenuata	OO - Reduced Fatty Acid Hydroperoxides
Prosystemin - Donor: Nicotiana attenuata	OO - Reduced Green Leaf Volatiles
Protease inhibitor - Donor: Nicotiana attenuata	OO - Reduced Growth
Pto-responsive gene 1 - Donor: Nicotiana attenuata	OO - Reduced Hydrogen Peroxide After Herbivore Attack
Putrescine N-methyl transferase - Donor: Nicotiana attenuata	OO - Reduced Jasmonate Signaling
R2R3 MYB Transcription Factor - Donor: Nicotiana attenuata	OO - Reduced Jasmonic Acid And Isoleucine Production
RE gene (RE065) - Donor: Nicotiana attenuata	OO - Reduced Jasmonic Acid Levels - Reduced Herbivore R
RNA dependent RNA polymerase 1 - Donor: Nicotiana attenuata	OO - Reduced Jasmonic Acid-Isoleucine
RNA dependent RNA polymerase 1 (RdR1) - Donor: Nicotiana attenuata	OO - Reduced Lipid Transferase
RNA dependent RNA polymerase 2 - Donor: Nicotiana attenuata	OO - Reduced Micrornas
RNA dependent RNA polymerase 2 (RdR2) - Donor: Nicotiana attenuata	OO - Reduced Micrornas And Phytohormone Responses To In
RNA-dependent RNA polymerase 3 - Donor: Nicotiana attenuata	OO - Reduced Micrornas And Reduced Phytohormone Respons
Rapid alkalization factor - Donor: Nicotiana attenuata	OO - Reduced Micrornas And Reduced Sensitivity To Jasmo
RuBPCase (Rubisco) - Donor: Arabidopsis thaliana	OO - Reduced Nicotine
RuBPCase (Rubisco) - Donor: Nicotiana attenuata	OO - Reduced Nicotine And Benzylacetone Emissions From
Rubisco activase - Donor: Nicotiana attenuata	OO - Reduced Nicotine And Ethylene Sensitive
S-adenosyl-L-methionine:jasmonic acid - Donor: Arabidopsis thaliana	OO - Reduced Nicotine And Proteinase Inhibitors
S-adenosyl-L-methionine:salicylic acid - Donor: Nicotiana tabacum	OO - Reduced Nitrate Reductase
SA inducible protein kinase - Donor: Nicotiana attenuata	OO - Reduced Pathogen Resistance
Salicylic Acid inducible kinase 2 - Donor: Nicotiana attenuata	OO - Reduced Pathogen Resistance And Decreased Phytohor

Salicylic Acid inducible protein kinase - Donor: Nicotiana attenuata	OO - Reduced Pathogen Resistance And Reduced Micrornas
Shaggy kinase - Donor: Nicotiana attenuata	OO - Reduced Pathogen Resistance And Reduced Sensitivit
Streptothricin acetyltransferase - Donor: Escherichia coli	OO - Reduced Pathogen Resistance, Reduced Nicotine, And
Streptothricin acetyltransferase* - Donor: Escherichia coli	OO - Reduced Pathogen Resistance, Reduced Volatiles, An
Transcription factor WRKY3 - Donor: Nicotiana attenuata	OO - Reduced Pathogene Response
Transcription factor WRKY6 - Donor: Nicotiana attenuata	OO - Reduced Pectin Methyl Esterase
Transferase - Donor: Nicotiana attenuata	OO - Reduced Photosynthetic Rates
Wound responsive kinase - Donor: Nicotiana attenuata	OO - Reduced Photosynthetic Rates And Increased Photosy
Xyloglucan endo-transglycosidase - Donor: Nicotiana attenuata	OO - Reduced Photosynthetic Rates And Reduced Sensitivi
Xyloglucan endo-transglycosidase 1 - Donor: Nicotiana attenuata	OO - Reduced Photosynthetic Rates, Reduced Nicotine, An
ZIM-type repressor protein - Donor: Nicotiana attenuata	OO - Reduced Photosynthetic Rates, Reduced Volatiles, A
ZIM-type repressor protein 3 end - Donor: Nicotiana attenuata	OO - Reduced Phytohormone Responses To Insect Attack
ZIM-type repressor protein 5 end - Donor: Nicotiana attenuata	OO - Reduced Phytohormone Responses To Insect Attack An
acetyl CoA carboxylase - Donor: Nicotiana attenuata	OO - Reduced Phytohormone Responses To Insect Attack, R
acyl-CoA oxidase - Donor: Nicotiana attenuata	OO - Reduced Proteinase Inhibitors
alpha-dioxygenase - Donor: Nicotiana attenuata	OO - Reduced Proteinase Inhibitors And Reduced Volatile
beta-glucuronidase (GusA)-marker gene - Donor: Escherichia coli	OO - Reduced Proteinase Inhibitors, Reduced Volatiles,
beta-subunit (gal83) of sucrose non fermenting pro - Donor: Nicotiana attenuata	OO - Reduced Rna-Dependent Reverse Polymerase
calmodulin-binding protein-like - Donor: Nicotiana attenuata	OO - Reduced Salicylic Acid And Reduced Phytohormone Re
ethylene receptor mutant (etr1-1) - Donor: Arabidopsis thaliana	OO - Reduced Salicylic Acid Levels - Reduced Pathogen R
gamma amino butyryl transaminase - Donor: Nicotiana attenuata	OO - Reduced Salicylic Acid Response
gene for pathogene resistance - Donor: Nicotiana attenuata	OO - Reduced Sensitivity To Jasmonates
jasmonic acid carboxyl methyltransferase (JMT) - Donor: Arabidopsis thaliana	OO - Reduced Sensitivity To Jasmonates And Reduced Phyt
jasmonic acid resistance protein (JAR4) - Donor: Nicotiana attenuata	OO - Reduced Systemin Polypeptide
jasmonic acid resistance protein (JAR6) - Donor: Nicotiana attenuata	OO - Reduced Thionin
jasmonic acid resistance protein 4 (JAR4) - Donor: Nicotiana attenuata	OO - Reduced Voc Synthesis And Diterpenoids
jasmonic acid resistance protein 6 (JAR6) - Donor: Nicotiana attenuata	OO - Reduced Volatiles
knotted-1 gene - Donor: Zea mays	OO - Reduced Volatiles And Constitutive/Elevated Bergam
lipoxygenase (LOX2) - Donor: Nicotiana attenuata	OO - Reduced Volatiles And Proteinase Inhibitors
lipoxygenase (LOX3) - Donor: Nicotiana attenuata	OO - Reduced Volatiles And Reduced Proteinase Inhibitor
non responding pathogen resistance proteine 1 - Donor: Nicotiana attenuata	OO - Reduced Volatiles, Reduced Nicotine, And Reduced P
non responding pathogene resistance proteine1 - Donor: Nicotiana attenuata	OO - Reduced Volatiles, Reduced Proteinase Inhibitors A

			none - Donor: none	OO - Reduced Volatiles, Reduced Proteinase Inhibitors,
			phytochrome B1 (PhyB1) - Donor: Solanum nigrum	OO - Reduced Volatiles, Reduces Proteinase Inhibitors,
			phytochrome B1 (PhyB1) - Donor: solanum nigrum	OO - Reduced Wound Responsive Kinase
			phytochrome B2 (PhyB2) - Donor: solanum nigrum	OO - Reduced Xyloglucan Endo-Transglycosidase
			plastitic aldolase - Donor: Nicotiana attenuata	OO - Reduced Xyloglucan Endo-Transglycosidase (Reduced
			utative giberellin beta-hydroxylase - Donor: N. attenuata	OO - Slight Shift In Ja Burst
			rubisco activase - Donor: Nicotiana attenuata	
			salicylate hydroxylase (NAHG1) - Donor: Nicotiana attenuata	
			salicylate hydroxylase (NAHG2) - Donor: Nicotiana attenuata	
			sedoheptulose-1, 7-bisphosphatase (SBPase) - Donor: Nicotiana tabacum	
			transcription factor WRKY3 - Donor: Nicotiana attenuata	
			transcription factor WRKY6 - Donor: Nicotiana attenuata	
			transcription factor wrky6 - Donor: Nicotiana attenuata	
			wound inducible protein kinase - Donor: N. attenuata	
06-242-01R	**Black nightshade**	Max Planck Institute for Chemical Ecology	1-aminocyclopropane 1-carboxylic acid oxidase - Donor: Solanum nigrum	OO - Altered Chlorophyll Production
			3-hydroxy-3-methylglutaryl-CoA-reductase - Donor: Solanum nigrum	OO - Altered Ethylene Response
			Calcium dependent protein kinase - Donor: S., nigrum	OO - Altered Kinase Signaling
			Coronatine insensitive 1 - Donor: Solanum nigrum	OO - Altered Kinase Signalling
			Ethylene receptor mutant (etr1-1) - Donor: A. thaliana	OO - Altered Oxylipin Signaling
			Hydroperoxide lyase - Donor: Solanum nigrum	OO - Altered Phenolic Profiles
			Jasmonic acid resistance protein 4 - Donor: S. nigrum	OO - Altered Terpenoid Profiles
			Jasmonic acid resistance protein 6 - Donor: S. nigrum	OO - Altered Volatile Emissions
			Lipoxygenase (LOX3) - Donor: Solanum nigrum	OO - Changed Ethylene Metabolism
			MAP kinase 2 - Donor: Solanum nigrum	OO - Changed Oxylipin Profiles
			MYC2 transcription factor - Donor: Solanum nigrum	OO - Constitutive Ethylene Response
			Pathogene resistance protein - Donor: Solanum nigrum	OO - Decreased Herbivore Resistance
			Phytochrome B1 - Donor: Solanum nigrum	OO - Decreased Pr1 A Pathogen Responsive Protein
			Phytochrome B2 - Donor: Solanum nigrum	OO - Decreased Protease Inhibitor Levels
			Prosystemin - Donor: Solanum nigrum	OO - Empty Vector Control
			Proteinase inhibitor - Donor: Solanum nigrum	OO - Reduced Alternative Oxidase
			Proteinase inhibitor 1 - Donor: Solanum nigrum	OO - Reduced Ja Responses
			Proteinase inhibitor 2 - Donor: Solanum nigrum	OO - Reduced Phytohormone Responses To Insect Attack
			Proteinase inhibitor1 - Donor: Solanum nigrum	OO - Reduced Proteinase Inhibitors
			Transcription factor WRKY3 - Donor: Nicotiana attenuata	OO - Reduced Sensitivity To Jasmonic Acid
			Transcription factor WRKY6 - Donor: Nicotiana attenuata	OO - Reduced Volatiles

				alpha-dioxygenase - Donor: Solanum nigrum alternative oxidase - Donor: Solanum nigrum chorismate synthase - Donor: Solanum nigrum constitutive triple response - Donor: Solanum nigrum ethylene receptor mutant - Donor: Solanum nigrum leucine aminopeptidase - Donor: Solanum nigrum	
06-226-104N	**Oilseed rape/ field mustard x oilseed rape hybrids**	University of Tennessee		Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki GFP - Donor: Aequorea victoria acetohydroxyacid synthase (ahas) - Donor: A. thaliana geberellic acid insensitive (delta gai) - Donor: A. thaliana mGFP5-ER - Donor: Aequorea, Arabidopsis nptii - Donor: Escherichia coli nptii* - Donor: Escherichia colo	HT - Sulfonylurea Tolerant IR - Corn Earworm Resistant MG - Visual Marker AP - Dwarfed
06-226-103N	**Oilseed rape/ field mustard**	University of Tennessee		mGFP5-ER - Donor: Aequorea, Arabidopsis nptii* - Donor: Escherichia colo nptii - Donor: Escherichia coli Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki GFP - Donor: Aequorea victoria acetohydroxyacid synthase (ahas) - Donor: A. thaliana geberellic acid insensitive (delta gai) - Donor: A. thaliana	MG - Visual Marker IR - Corn Earworm Resistant HT - Sulfonylurea Tolerant AP - Dwarfed
06-221-01R	**Bahiagrass**	University of Florida		ArgE - Donor: Escherichia coli C-repeat binding factor x3 (CFB3) - Donor: Hordeum spontaneum Dehydrin 1 (Dhn1) - Donor: Hordeum spontaneum Dhn5 - Donor: Hordeum spontaneum Dhn8 - Donor: Hordeum spontaneum OsDREB1A - Donor: Oryza sativa Osmyb4 - Donor: Oryza sativa WRYK8 - Donor: Hordeum vulgare nptII gene* - Donor: Escherichia coli	AP - Cold Tolerance Increased AP - Drought Tolerance Increased AP - Dwarfed AP - Salt Tolerance Increased
06-219-01R	**Dwarf bahiagrass**	University of Florida		CBF3 - C-repeat binding factor x3 - Donor: Hordeum spontaneum	OO - Dwarfism PQ - Drought Tolerance PQ - Salt And Cold Tolerance
06-219-104N	**Oilseed rape**	University of Tennessee		Bt Cry1Ac - Donor: Bacillus thuringiensis var kurstaki GFP - Donor: Aequorea victoria acetohydroxyacid synthase (ahas) - Donor: A. thaliana geberellic acid insensitive (delta gai) - Donor: A. thaliana mGFP5-ER - Donor: Aequorea, Arabidopsis	MG - Visual Marker IR - Corn Earworm Resistant HT - Sulfonylurea Tolerant AP - Dwarfed

			nptii - Donor: Escherichia coli nptii* - Donor: Escherichia colo	
06-201-01R	**Festuca arundinacea**	North Carolina State University	vacuolar pyrophosphatase - Donor: Arabidopsis thaliana	AP - Drought Tolerant
06-167-102N	**Tomato**	Purdue University	SOS1 - Donor: Arabidopsis thaliana hpt* - Donor: Escherichia coli	AP - Salt Tolerance Increased
06-150-101N	**Potato**	Michigan State University	ADP-glucose pyrophosphorylase - Donor: Escherichia coli Cry1Ia1 - Donor: Bacillus thuringiensis var kurstaki Cry3a - Donor: Bacillus thuringiensis var tenebrionis RB - Donor: Solanum bulbocastanum proteinase inhibitor I, avidin - Donor: potato, chicken transcription factor - Donor: Arabidopsis thaliana NPT II* - Donor: Escherichia coli	PQ - Starch Level Increased IR - Lepidopteran/Coleopteran Resistance IR - Coleopteran Resistance FR - Phytophthora Infestans Resistance AP - Cold/Drought Resistance
06-145-109N	**Rice**	Cold Spring Harbor Laboratory	trehalose-6-phosphate phosphatase - Donor: O. sativa hygromycin phosphotransferase* - Donor: E.coli	AP - Altered Plant Development
06-139-106N	**Tomato**	Purdue University	Mitochondrial small heat shock protein AB017134 - Donor: Lycopersicon esculentum Partial Catalase (AF112368) antisense orientation - Donor: Lycopersicon esculentum S-adenosyl methionine decarboxylase Acce M38434 - Donor: Saccharomyces cerevisiae Spermidine synthase (SPE3) gene (Accession U27519 - Donor: Saccharomyces cerevisiae Vis1 a sHSP (Acce. AY128102) - Donor: Lyc. esculentum neomycin phosphotransferase (npt)* - Donor: E. coli neomycin phosphotransferase* - Donor: Escherichia coli	PQ - Environmental Stress Response PQ - Altered Oxidative Stress Response PQ - Fruit Ripening Altered PQ - Higher Polyamine Levels PQ - Altered Heat Stress Response
06-137-117N	**Grey poplar**	Oregon State University	GAI - Donor: Arabidopsis thaliana Mutated GAI - Donor: Arabidopsis thaliana PcGA2Ox1 - Donor: Phaseolus coccineus PtaGA2Ox1 - Donor: Populus tremula x P. alba mutated rgl-1 - Donor: Arabidopsis thaliana NPT I* - Donor: Corynebacterium diphtheriae NPTII* - Donor: Escherichia coli nptI* - Donor: Corynebacterium diphtheriae	OO - Reduced Stature (Dwarfing)
06-121-103N	**Apple**	Cornell University	uidA - Donor: Escherichia coli kanamycin phosphotransferase* - Donor: Escherichia coli S6PDH - Donor: Malus domestica	PQ - Down-Regulation Of Sorbitol Synthesis
06-107-103N	**Cotton**	Texas Tech	Endoxyloglucan transferase - Donor: Gossypium hirsutum	AP - Stress Tolerancefiber Quality

		University	Glutathione reductase - Donor: Arabidopsis thaliana Catalase 3 - Donor: Zea mays Ascorbate peroxidase - Donor: Pisum sativum neomycin phosphotransferase II* - Donor: E. coli	AP - Stress Tolerance
06-107-102N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pisum sativum Catalase 3 - Donor: Zea mays Endoxyloglucan transferase - Donor: Gossypium hirsutum Glutathione reductase - Donor: Arabidopsis thaliana neomycin phosphotransferase II* - Donor: E. coli	AP - Stress Tolerancefiber Quality AP - Stress Tolerance
06-090-20N	**Rice**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley Beta-glucuronidase - Donor: E. coli Hygromycin phosphotransferase*	AP - Nitrogen Utilization Efficiency Increase
06-088-01R	**Tobacco**	Edenspace Systems Corporation	B-1,4-endoglucanase - Donor: Acidothermus cellulolyticus Flowering locus C - Donor: Arabidopsis thaliana NptII* Phosphinothricin acetyl transferase*	AP - Flowering Time Altered OO - Increased Cellulose Hydrolysis
06-087-12N	**Corn**	Cold Spring Harbor Laboratory	Fasciated ear2 - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Yield Increased
06-087-11N	**Rapeseed**	Arcadia Biosciences	CBI - Donor: CBI CBI* Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Utilization Efficiency Increase
06-086-11N	**Corn**	University of Illinois	B-glucuronidase - Donor: E. coli Glossy15 - Donor: Corn NptII*	MG - Visual Marker OO - Epidermal Cells Increased On Juvenile Leaves
06-082-08N	**Rice**	Arcadia Biosciences	Sodium/hydrogen ion exchanger - Donor: Arab. thaliana Hygromycin phosphotransferase*	AP - Salt Tolerance Increased
06-080-05N	**Cotton**	Texas Tech University	Proton transporter - Donor: Arab. thaliana NptII*	AP - Drought Tolerant
06-076-02N	**Populus tremula x P. alba**	Mississippi State University	CONSTANS - Donor: Populus deltoides NptII*	OO - Flowering Altered
06-076-01N	**Populus tremula x P. alba**	Mississippi State University	APETELA1 - Donor: Populus deltoides Abcissic acid insensitive - Donor: Populus deltoides Gibberalic acid insensitive - Donor: Populus deltoides Phytochrome A - Donor: Populus deltoides Phytochrome B - Donor: Populus deltoides	OO - Flowering Altered

			Suppression of overexpression of constans - Donor: Populus deltoides NptII*	
06-073-07N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
06-069-05N	**Populus tremula x P. alba**	Oregon State University	Gibberellin 2 oxidase - Donor: Phaselous coccineus Repressor of gibberalin response - Donor: A. thaliana NptII*	OO - Dwarfed
06-019-04R	**Corn**	Pioneer Hi-Bred International, Inc.	Adenine methylase - Donor: E. coli B-glucuronidase - Donor: E. coli CBI - Donor: Barley CBI - Donor: Wheat CBI - Donor: Soybean CBI - Donor: Rice CBI - Donor: E. coli CBI - Donor: Corn CBI - Donor: CBI CBI - Donor: Bt CBI - Donor: Arab. thaliana Cry1F - Donor: Bt Dihydrodipicolinate synthase - Donor: Corynebacterium glutamicum Glyphosate N-acetyltransferase - Donor: Bacillus lichenformes Glyphosate N-acetyltransferase* High sulfur zein - Donor: Corn Hygromycin phosphotransferase - Donor: E. coli Luciferase - Donor: Photinus pyralis Lysine ketoglutarate reductase - Donor: Corn MyB transcription factor - Donor: Corn Phosphinothricin acetyl transferase* Phosphinothricin acetyl transferase - Donor: Streptoalloteichus hindustanus Phosphinothricin acetyl transferase - Donor: Strep. hygroscopicus	AP - Fertility Altered AP - Growth Rate Reduced AP - Maturity Altered AP - Nitrogen Utilization Efficiency Increase AP - Yield Increased AP - Yield Stability Increased FR - Ear Mold Resistant HT - CBI HT - Glyphosate Tolerant HT - Phosphinothricin Tolerant IR - Coleopteran Resistant IR - Lepidopteran Resistant MG - Selectable Marker MG - Visual Marker OO - CBI OO - Increased Transformation Frequency PQ - Altered Amino Acid Composition PQ - Altered Starch Content PQ - Animal Feed Quality Improved PQ - Cell Wall Altered PQ - Digestibility Improved PQ - Fatty Acid Level Altered PQ - Feed Properties Altered PQ - Fiber Quality Altered PQ - Grain Processing Improved PQ - Lipid Profile Altered PQ - Lysine Level Increased PQ - Nutritional Quality Improved

				PQ - Oil Profile Altered PQ - Protein Altered PQ - Seed Oil Content Increased PQ - Starch Metabolism Altered
06-019-02R	**Soybean**	Pioneer Hi-Bred International, Inc.	Acetolactate synthase - Donor: Arabidopsis thaliana Acyl-ACP thioesterase - Donor: Soybean Acyl-ACP thioesterase - Donor: Rapeseed Aspartokinase II-homoserine dehydrogenase - Donor: E. coli B-glucuronidase - Donor: E. coli Beta-conglycinin (alpha subunit) - Donor: Soybean CBI - Donor: Alfalfa CBI - Donor: Barley CBI - Donor: CBI CBI - Donor: Corn CBI - Donor: Euphorbia lagascae CBI - Donor: Mortierella alpina CBI - Donor: Parthenium argentatum CBI - Donor: Rice CBI - Donor: Schizochytrium aggregatum CBI - Donor: Soybean CBI - Donor: Sunflower CBI - Donor: Vernonia galamensis CBI - Donor: Wheat CBI* Cystathionine synthase - Donor: Soybean Delta-12 desaturase - Donor: Soybean Delta-9 desaturase - Donor: Soybean Glycinin I - Donor: Soybean Glycinin I exon 4 - Donor: Soybean Glycinin IV exon 3 - Donor: Soybean Glyphosate N-acetyltransferase - Donor: Bacillus lichenformes High sulfur zein - Donor: Corn Hygromycin phosphotransferase - Donor: E. coli Luciferase - Donor: Photinus pyralis MyB transcription factor - Donor: Corn NptII - Donor: E. coli Omega 3 desaturase - Donor: Soybean	AP - Altered Stem Attributes AP - Maturity Altered AP - Yield Increased AP - Yield Stability Increased FR - CBI FR - Sclerotinia Resistant HT - CBI HT - Glyphosate Tolerant HT - Phosphinothricin Tolerant HT - Sulfonylurea Tolerant IR - Lepidopteran Resistant IR - Soybean Aphid Resistant MG - Selectable Marker MG - Visual Marker NR - CBI OO - Increased Transformation Frequency OO - Recombinase Produced PQ - Altered Amino Acid Composition PQ - Carbohydrate Metabolism Altered PQ - Cell Wall Altered PQ - Fatty Acid Level Altered PQ - Feed Properties Altered PQ - Fiber Quality Altered PQ - Flavinoid Level Altered PQ - Food Quality Altered PQ - Lipid Profile Altered PQ - Oil Quality Altered PQ - Phytate Reduced PQ - Protein Quality Altered PQ - Seed Oil Content Increased PQ - Starch Metabolism Altered

			Omega 6 desaturase - Donor: Soybean Phosphinothricin acetyltransferase - Donor: Strep. viridochromogenes Storage protein - Donor: Corn	
06-019-01R	**Soybean**	Pioneer Hi-Bred International, Inc.	Acetolactate synthase - Donor: Arabidopsis thaliana Acyl-ACP thioesterase - Donor: Soybean Acyl-ACP thioesterase - Donor: Rapeseed Aspartokinase II-homoserine dehydrogenase - Donor: E. coli B-glucuronidase - Donor: E. coli Beta-conglycinin (alpha subunit) - Donor: Soybean CBI - Donor: Wheat CBI* CBI - Donor: Sunflower CBI - Donor: Vernonia galamensis CBI - Donor: Soybean CBI - Donor: Schizochytrium aggregatum CBI - Donor: Rice CBI - Donor: Parthenium argentatum CBI - Donor: Alfalfa CBI - Donor: Arab. thaliana CBI - Donor: Barley CBI - Donor: CBI CBI - Donor: Corn CBI - Donor: Euphorbia lagascae CBI - Donor: Mortierella alpina Cystathionine synthase - Donor: Soybean Delta-12 desaturase - Donor: Soybean Delta-9 desaturase - Donor: Soybean Glycinin I - Donor: Soybean Glycinin I exon 4 - Donor: Soybean Glycinin IV exon 3 - Donor: Soybean Glyphosate N-acetyltransferase - Donor: Bacillus lichenformes High sulfur zein - Donor: Corn Hygromycin phosphotransferase* - Donor: E. coli Luciferase - Donor: Photinus pyralis MyB transcription factor - Donor: Corn NptII* - Donor: E. coli Omega 3 desaturase - Donor: Soybean	AP - Altered Stem Attributes AP - Maturity Altered AP - Yield Increased AP - Yield Stability Increased FR - CBI FR - Sclerotinia Resistant HT - CBI HT - Glyphosate Tolerant HT - Phosphinothricin Tolerant HT - Sulfonylurea Tolerant IR - Lepidopteran Resistant IR - Soybean Aphid Resistant MG - Selectable Marker MG - Visual Marker NR - CBI OO - Increased Transformation Frequency OO - Recombinase Produced PQ - Altered Amino Acid Composition PQ - Carbohydrate Metabolism Altered PQ - Cell Wall Altered PQ - Fatty Acid Level Altered PQ - Feed Properties Altered PQ - Fiber Quality Altered PQ - Flavinoid Level Altered PQ - Food Quality Altered PQ - Lipid Profile Altered PQ - Oil Quality Altered PQ - Phytate Reduced PQ - Protein Quality Altered PQ - Seed Oil Content Increased PQ - Starch Metabolism Altered

			Omega 6 desaturase - Donor: Soybean Phosphinothricin acetyltransferase - Donor: Strep. viridochromogenes Storage protein - Donor: Corn	
06-018-12N	**Corn**	University of Wisconsin/Madison	Teosinte glume architecture 1 - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Flowering Altered
06-018-01N	**Wheat**	Oklahoma State University	Mannitol dehydrogenase - Donor: E. coli Phosphinothricin acetyl transferase*	AP - Drought Tolerant
06-003-08N	**Nicotiana attenuata**	Max Planck Institute for Chemical Ecology	RNA dependent RNA polymerase - Donor: N. attenuata Pectin methylesterase - Donor: Nicotania attenuata Hygromycin phosphotransferase* Ribulose-1,5-bisphosphate carboxylase activase - Donor: Nicotania attenuata Putrescine-N-methyltransferase - Donor: N. attenuata Prosystemin - Donor: Nicotania attenuata Lipoxygenase - Donor: Nicotania attenuata Hydroperoxide lyase - Donor: Nicotania attenuata Ethylene receptor protein - Donor: Arab. thaliana alternative oxidase 3 - Donor: Nicotania attenuata Aminocyclopropane carboxylicacid oxidase - Donor: Nicotania attenuata Alpha-dioxygenase - Donor: Nicotania attenuata Allene oxide synthase - Donor: Nicotania attenuata Pathogenesis protein 1a - Donor: Nicotania attenuata	OO - Empty Transformation Vector OO - Growth Rate Altered OO - Lepidopteran Resistant
05-364-01R	**Bahiagrass**	University of Florida	ATH1 floral gene inhibitor - Donor: Arabidopsis thaliana C-repeat binding factor (CBF) - Donor: Hordeum spontaneum Gibberellin 2 oxidase - Donor: Arabidopsis thaliana NptII* Phytochrome photoreceptor - Donor: Oat Transcription factor for ATHB16 - HDZIP - Donor: Arabidopsis thaliana Transcription factor for WRKY38 receptor kinase - Donor: Barley	PQ - Dwarfed
05-341-04N	**Populus tremula x P. tremuloides**	University of Connecticut	Gibberellin 20 oxidase - Donor: Cotton NptII*	AP - Growth Rate Altered
05-312-01R	**Festuca arundinacea**	North Carolina State University	Dermaseptin B - Donor: Phyllomedusa bicolor Hygromycin phosphotransferase* Lysozyme - Donor: Bacteriophage T4 Pi9 blast resistance gene - Donor: Rice	AP - Drought Tolerant FR - Brown Patch Resistant FR - Gray Lead Spot Resistant

			Vacuolar pyrophosphatase - Donor: Arabidopsis thaliana	
05-305-07N	**Soybean**	University of Nebraska/Lincoln	Ferric chelate reductase - Donor: Arabidopsis thaliana Phosphinothricin acetyl transferase*	AP - Environmental Stress Reduced
05-298-02N	**Cotton**	United States Department of Agriculture/ Agricultur	Ascorbate peroxidase - Donor: Pea Catalase - Donor: Corn Glutathione reductase - Donor: Arab. thaliana Superoxide dismutase - Donor: Nicotiana plumbaginifolia NptII*	AP - Environmental Stress Reduced
05-294-01R	**Bahiagrass**	University of Florida	C-repeat binding factor (CBF) - Donor: Barley Dehydrin1 - Donor: Barley Dehydrin5 - Donor: Barley Dehydrin8 - Donor: Barley N-acetylorthininase - Donor: E. coli NptII* Transcription factor for OsDREBIA - Donor: Rice Transcription factor for Osmyb4 - Donor: Rice Transcription factor for WRKY38 receptor kinase - Donor: Barley	PQ - Cold Tolerant PQ - Drought Tolerant PQ - Salt Tolerance Increased
05-262-02N	**Rapeseed**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Utilization Efficiency Increase
05-245-05N	**Rapeseed**	University of Tennessee	NptII* Acetohydroxyacid synthase variant - Donor: A. thaliana Repressor of gibberalin response - Donor: A. thaliana	HT - Dwarfed
05-245-04N	**Rapeseed**	University of Tennessee	Acetohydroxyacid synthase variant - Donor: Arab. thaliana Repressor of gibberalin response - Donor: A. thaliana NptII*	HT - Dwarfed
05-195-06N	**Corn**	University of Florida	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
05-192-14N	**Populus tremula x P. alba**	University of Connecticut	Phosphinothricin acetyl transferase* Vacuolar pyrophosphatase - Donor: Arab. thaliana	OO - Drought Tolerant
05-158-02N	**Poplar**	Oregon State University	Gibberalic acid insensitive - Donor: Arab. thaliana NptII*	OO - Dwarfed
05-152-02N	**Tomato**	Purdue University	Sodium/hydrogen ion exchanger - Donor: Arabidopsis NptII*	AP - Environmental Stress Reduced
05-138-25N	**Potato**	Michigan State University	DNA binding factor 1 (CRT/DRE)(CFB1 gene) - Donor: Arabidopsis NptII*	PQ - Cold Tolerant PQ - Drought Tolerant
05-130-07N	**Cucumber**	Michigan State	Cold regulated gene binding factor (CBF) - Donor: Arabidopsis	AP - Salt Tolerance Increased

		University	NptII*	
05-130-01N	**Poplar**	Oregon State University	Gibberellin 20 oxidase - Donor: Poplar NptII*	OO - Dwarfed
05-117-10N	**Corn**	Cold Spring Harbor Laboratory	Fasciated ear2 - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Altered Plant Development
05-117-09N	**Corn**	Cold Spring Harbor Laboratory	ABPHYL1 - Donor: Corn Yello fluorescent protein - Donor: Jellyfish Phosphinothricin acetyl transferase*	OO - Modified Growth Characteristics
05-112-01N	**Corn**	University of Wisconsin/Madison	Agamous-like gene 1 - Donor: Corn Floricaula leafy 2 - Donor: Corn Teosinte branched 1 - Donor: Corn Teosinte glume architecture 1 - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Flowering Altered
05-088-03N	**Rice**	Arcadia Biosciences	Sodium/hydrogen ion exchanger - Donor: Arab. thaliana Hygromycin phosphotransferase*	AP - Salt Tolerance Increased
05-084-04N	**Corn**	University of Nebraska/Lincoln	Heat shock protein - Donor: Corn alternative oxidase 3 - Donor: Corn NptII*	OO - Heat Tolerant
05-076-11N	**Bahiagrass**	University of Florida	C-repeat binding factor (CBF) - Donor: Hordeum N-acetyl glucosidase - Donor: E. coli NptII*	AP - Cold Tolerant AP - Salt Tolerance Increased
05-066-09N	**Cotton**	Texas Tech University	Catalase antisense - Donor: Corn Endoxyloglucan transferase - Donor: Cotton Glutathione reductase - Donor: Arabidopsis NptII* Ascorbate peroxidase - Donor: Pea	AP - Environmental Stress Reduced
05-066-08N	**Cotton**	Texas Tech University	NptII* Glutathione reductase - Donor: Arabidopsis Catalase antisense - Donor: Corn Ascorbate peroxidase - Donor: Pea Endoxyloglucan transferase - Donor: Cotton	AP - Environmental Stress Reduced
05-066-07N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Catalase antisense - Donor: Corn Endoxyloglucan transferase - Donor: Cotton Glutathione reductase - Donor: Arabidopsis NptII*	AP - Environmental Stress Reduced
05-066-06N	**Cotton**	Texas Tech	Sodium/hydrogen ion exchanger - Donor: Arabidopsis	AP - Environmental Stress Reduced

		University	NptII*	
05-066-05N	**Cotton**	Texas Tech University	Catalase antisense - Donor: Corn NptII* Endoxyloglucan transferase - Donor: Cotton Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arabidopsis	AP - Environmental Stress Reduced
05-049-05N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
05-049-04N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
05-049-26N	**Corn**	University of Illinois	B-glucuronidase - Donor: E. coli Homeotic regulatory gene (glossy 15) - Donor: Corn NptII*	OO - Epidermal Cells Increased On Juvenile Leaves
05-038-01N	**Cotton**	Arcadia Biosciences	Metal ion/hydrogen ion antiporter - Donor: Arab. thaliana NptII*	AP - Salt Tolerance Increased
05-032-12N	**Wheat**	University of Nebraska/Lincoln	NptII* Adenosine diphosphoglucose pyrophosphorylase - Donor: Corn	AP - Yield Increased
05-026-25N	**Wheat**	Oklahoma State University	Mannitol dehydrogenase - Donor: E. coli Phosphinothricin acetyl transferase*	AP - Drought Tolerant
04-362-13N	**Petunia**	University of Florida	Cold regulated gene binding factor (CBF) - Donor: Arab. thaliana	PQ - Cold Tolerant PQ - Drought Tolerant PQ - Salt Tolerance Increased
04-355-01N	**Tomato**	Arcadia Biosciences	Sodium/hydrogen ion exchanger - Donor: Arab. thaliana NptII*	AP - Salt Tolerance Increased
04-274-01N	**Rapeseed**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Metabolism Altered
04-191-06N	**Corn**	University of Minnesota	Isopentenyl transferase - Donor: Agro. tumefaciens Phosphinothricin acetyl transferase*	AP - Yield Increased
04-145-02N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Catalase - Donor: Corn Glutathione reductase - Donor: Arabidopsis NptII*	AP - Environmental Stress Reduced
04-129-06N	**Cucumber**	Michigan State University	C-repeat binding factor (CBF) - Donor: Arabidopsis NptII*	AP - Salt Tolerance Increased
04-110-09N	**Creeping bentgrass**	Kansas State University	Late embryogenesis abundant protein - Donor: Barley Thaumatin related protein - Donor: Rice Phosphinothricin acetyl transferase*	AP - Drought Tolerant

04-106-01N	**Wheat**	Oklahoma State University	Mannitol dehydrogenase - Donor: E. coli Phosphinothricin acetyl transferase*	AP - Drought Tolerant
04-098-08N	**Rapeseed**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Metabolism Altered
04-098-07N	**Poplar**	Mississippi State University	CONSTANS - Donor: Populus deltoides NptII*	OO - Flowering Time Altered
04-096-04N	**Poplar**	Oregon State University	Phytochrome B - Donor: Poplar NptII*	OO - Light Response Altered
04-069-04N	**Rice**	Louisiana State University	ADP-glucose pyrophosphorylase - Donor: Corn Hygromycin phosphotransferase - Donor: E. coli	AP - Yield Increased
04-063-01N	**Corn**	University of Illinois	Homeotic regulatory gene (glossy 15) - Donor: Corn	OO - Epidermal Cells Increased On Juvenile Leaves
04-044-12N	**Wheat**	Montana State University	ADP glucose pyrophosphorylase - Donor: Corn ADP glucose pyrophosphorylase - Donor: Potato Phosphinothricin acetyl transferase*	AP - Yield Increased
04-042-11N	**Wheat**	University of Nebraska/Lincoln	NptII* Adenosine diphosphoglucose pyrophosphorylase - Donor: Corn	PQ - Yield Increased
04-033-18N	**Wheat**	Montana State University	Phosphinothricin acetyl transferase* ADP-glucose pyrophosphorylase - Donor: Corn	AP - Yield Increased
04-033-17N	**Wheat**	Montana State University	Phytochrome b - Donor: Arab. thaliana Phosphinothricin acetyl transferase*	AP - Yield Increased
04-033-20N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
04-009-01R	**Brassica juncea**	United States of Department Agriculture/ Agricultur	TP sulfurylase - Donor: Arab. thaliana Cystathionine synthase - Donor: Arab. thaliana Glutamylcysteine synthetase - Donor: E. coli Hygromycin phosphotransferase* Methionine methyltransferase - Donor: Arab. thaliana NptII* SMT sulfurylase - Donor: Astragalus bisulcatus Selenocystine lyase - Donor: Mouse Sodium/hydrogen ion exchanger - Donor: Arab. thaliana	AP - Salt Tolerance Increased OO - Industrial Enzyme Produced
03-276-04N	**Rapeseed**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Metabolism Altered
03-118-02N	**Cucumber**	Michigan State University	C-repeat binding factor (CBF) - Donor: Arabadopsis NptII*	AP - Salt Tolerance Increased
03-111-06N	**Tobacco**	University of Hawaii	Violaxanthin de-epoxidase antisense - Donor: Tobacco	AP - Photosynthesis Enchanced

			Violaxanthin de-epoxidase - Donor: Arab. thaliana Violaxanthin de-epoxidase - Donor: Lettuce NptII*	
03-105-01N	**Rapeseed**	Arcadia Biosciences	Alanine aminotransferase - Donor: Barley NptII*	AP - Nitrogen Metabolism Altered
03-078-12N	**Rice**	Louisiana State University	ADP-glucose pyrophosphorylase - Donor: Corn Hygromycin phosphotransferase*	AP - Yield Increased
03-077-09N	**Tobacco**	University of Kentucky	S-adenosylmethione hydrolase - Donor: Bacteriophage T3 NptII*	OO - Germination Increased
03-042-20N	**Corn**	University of Illinois	Homeotic regulatory gene (glossy 15) - Donor: Corn NptII*	OO - Epidermal Cells Increased On Juvenile Leaves
03-034-04N	**Wheat**	Montana State University	Phosphinothricin acetyl transferase* ADP-glucose pyrophosphorylase - Donor: Corn	AP - Yield Increased
03-034-09N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
03-034-08N	**Wheat**	Montana State University	Invertase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
03-034-07N	**Wheat**	Montana State University	ADP glucose pyrophosphorylase - Donor: Corn ADP glucose pyrophosphorylase - Donor: Potato Phosphinothricin acetyl transferase*	AP - Yield Increased
03-034-06N	**Wheat**	Montana State University	Phytochrome b - Donor: Arab. thaliana Phosphinothricin acetyl transferase*	AP - Yield Increased
03-024-12N	**Wheat**	University of Nebraska/Lincoln	ADP glucose pyrophosphorylase - Donor: Corn NptII*	AP - Yield Increased
02-361-02N	**Creeping bentgrass**	Kansas State University	Thaumatin related protein - Donor: Rice Phosphinothricin acetyl transferase* Late embryogenesis abundant protein - Donor: Barley	AP - Drought Tolerant
02-165-02N	**Tobacco**	University of Hawaii	Violaxanthin de-epoxidase - Donor: Tobacco NptII* Violaxanthin de-epoxidase - Donor: Lettuce	AP - Growth Rate Altered
02-151-10N	**Tomato**	CBI	Transcription factor for HY5 gene silenced - Donor: Tomato NptII*	PQ - Pigment Composition Altered PQ - Dwarfed
02-151-12N	**Tomato**	CBI	Transcription factor for DET1 gene silenced - Donor: Tomato NptII*	PQ - Pigment Composition Altered PQ - Dwarfed
02-151-11N	**Tomato**	CBI	NptII*	PQ - Dwarfed

			Transcription factor for COP gene silenced - Donor: Tomato	PQ - Pigment Composition Altered
02-137-01N	**Cucumber**	Michigan State University	C-repeat binding factor (CBF) - Donor: Arabadopsis NptII*	AP - Salt Tolerance Increased
02-113-06N	**Wheat**	Kansas State University	Late embryogenesis abundant protein - Donor: Barley Phosphinothricin acetyl transferase*	AP - Drought Tolerant
02-105-07N	**Tomato**	Oregon State University	Ascorbate peroxidase - Donor: Pea NptII*	AP - Environmental Stress Reduced
02-099-12N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arab. thaliana NptII*	AP - Environmental Stress Reduced
02-093-10N	**Tomato**	Purdue University	Heat shock protein - Donor: Tomato NptII*	PQ - Heat Tolerant
02-088-07N	**Wheat**	University of Nebraska/Lincoln	ADP glucose pyrophosphorylase - Donor: Corn NptII*	PQ - Yield Increased
02-072-13N	**Corn**	University of Florida	ADP glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Seed Size Increase
02-070-04N	**Rice**	Louisiana State University	ADP-glucose pyrophosphorylase - Donor: Corn Hygromycin phosphotransferase - Donor: E. coli Hygromycin phosphotransferase*	AP - Yield Increased
02-058-04N	**Corn**	University of Florida	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Seed Weight Increased
02-044-09N	**Wheat**	Montana State University	Phytochrome b - Donor: Arab. thaliana Phosphinothricin acetyl transferase*	AP - Yield Increased
02-044-07N	**Wheat**	Montana State University	ADP glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased MG - Visual Marker
02-044-06N	**Wheat**	Montana State University	ADP glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
02-038-01N	**Corn**	University of California	Dehydroascorbate reductase - Donor: Wheat Phosphinothricin acetyl transferase*	AP - Environmental Stress Reduced
02-032-18N	**Corn**	University of Illinois	Homeotic regulatory gene (glossy 15) - Donor: Corn NptII*	OO - Epidermal Cells Increased On Juvenile Leaves
02-022-61N	**Wheat**	University of Nebraska/Lincoln	ADP glucose pyrophosphorylase - Donor: Corn	PQ - Yield Increased
02-015-05N	**Creeping bentgrass**	Kansas State University	Late embryogenesis abundant protein - Donor: Barley Thaumatin related protein - Donor: Rice Phosphinothricin acetyl transferase*	AP - Drought Tolerant

01-353-05N	**Kentucky bluegrass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-353-02N	**Creeping bentgrass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-353-01N	**Creeping bentgrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
01-353-04N	**Kentucky bluegrass**	Rutgers University	Hygromycin phosphotransferase* Betaine aldehyde dehydrogenase - Donor: Garden orach	AP - Drought Tolerant
01-353-06N	**Kentucky bluegrass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-353-07N	**Perennial ryegrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-353-08N	**Bermuda-grass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
01-353-09N	**Bermuda-grass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
01-353-10N	**Bermuda-grass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-353-03N	**Creeping bentgrass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
01-241-01N	**Corn**	University of Florida	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	PQ - Seed Size Increase PQ - Visual Marker
01-138-03N	**Rice**	Louisiana State University	ADP-glucose pyrophosphorylase - Donor: Corn Hygromycin phosphotransferase*	AP - Yield Increased
01-130-03N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Arabidopsis NptII*	AP - Environmental Stress Reduced
01-122-07N	**Creeping bentgrass**	Rutgers University	Hygromycin phosphotransferase* Betaine aldehyde dehydrogenase - Donor: Garden orach	AP - Salt Tolerance Increased
01-074-15N	**Wheat**	Montana State University	ADP glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased
01-066-07N	**Rapeseed**	Mendel Biotechnology	Cold regulated gene binding factor (CBF) - Donor: Arab. thaliana NptII*	AP - Cold Tolerant
01-044-04N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arabadopsis NptII*	AP - Environmental Stress Reduced
01-023-27N	**Wheat**	Montana State University	ADP-glucose pyrophosphorylase - Donor: Corn Phosphinothricin acetyl transferase*	AP - Yield Increased AP - Starch Level Increased

00-322-04N	**Creeping bentgrass**	Rutgers University	Late embryogenesis abundant protein - Donor: Hordeum Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
00-322-03N	**Creeping bentgrass**	Rutgers University	Hygromycin phosphotransferase* Levansucrase - Donor: Bt	AP - Drought Tolerant AP - Salt Tolerance Increased
00-322-05N	**Kentucky bluegrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-322-07N	**Kentucky bluegrass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-322-08N	**Perennial ryegrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-322-09N	**Bermuda-grass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-322-10N	**Bermuda-grass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
00-322-11N	**Bermuda-grass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
00-322-06N	**Kentucky bluegrass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-322-02N	**Creeping bentgrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
00-277-02N	**Cotton**	Bowdoin College	Ascorbate peroxidase - Donor: Pea Superoxide dismutase - Donor: Nicotiana plumbaginifolia NptII*	AP - Drought Tolerant
00-271-01N	**Clary**	R J Reynolds	Leafy homeotic regulatory gene - Donor: Arab. thaliana NptII*	AP - Flowering Altered
00-115-03N	**Creeping bentgrass**	Rutgers University	Betaine aldehyde dehydrogenase - Donor: Garden orach Hygromycin phosphotransferase*	AP - Salt Tolerance Increased
00-115-02N	**Creeping bentgrass**	Rutgers University	Thiamine biosynthetic enzyme - Donor: Corn Hygromycin phosphotransferase*	AP - Heat Tolerant
00-115-09N	**Creeping bentgrass**	Rutgers University	Hygromycin phosphotransferase* Late embryogenesis abundant protein - Donor: Barley	AP - Salt Tolerance Increased
00-088-36N	**Wheat**	Montana State University	Late embryogenesis abundant protein - Donor: Barley Phosphinothricin acetyl transferase*	AP - Drought Tolerant
00-034-18N	**Walnut**	University of California	rol hormone gene - Donor: Agro. rhizogenes NptII*	AP - Adventious Root Formation Increased
99-326-01N	**Kentucky bluegrass**	Rutgers University	Late embryogenesis abundant protein - Donor: Barley Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant

99-308-04N	**Creeping bentgrass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Drought Tolerant AP - Salt Tolerance Increased
99-308-07N	**Kentucky bluegrass**	Rutgers University	Hygromycin phosphotransferase* Levansucrase - Donor: Bt	AP - Salt Tolerance Increased AP - Drought Tolerant
99-308-06N	**Kentucky bluegrass**	Rutgers University	Betaine aldehyde dehydrogenase Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
99-308-11N	**Bermuda-grass**	Rutgers University	Levansucrase - Donor: Bt Hygromycin phosphotransferase*	AP - Salt Tolerance Increased AP - Drought Tolerant
99-209-17N	**Tomato**	University of Florida	NptII* Agamous-like gene 8 - Donor: Arab. thaliana	PQ - Seed Size Increase
99-103-08N	**Creeping bentgrass**	Cook College Rutgers University	Thiamine biosynthetic enzyme - Donor: Corn Hygromycin phosphotransferase*	AP - Heat Tolerant
99-103-07N	**Creeping bentgrass**	Cook College Rutgers University	Hygromycin phosphotransferase* Citrate synthase	AP - Aluminum Tolerant
99-095-06N	**Cotton**	Texas Tech University	NptII* Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arab. thaliana	AP - Oxidative Stress Tolerant
99-075-11N	**Corn**	University of Florida	Sucrose synthase - Donor: Corn Phosphinothricin acetyl transferase*	OO - Seed Weight Increased
99-075-12N	**Corn**	University of Florida	Phosphinothricin acetyl transferase* ADP glucose pyrophosphorylase - Donor: Corn	PQ - Seed Weight Increased
99-061-17N	**Cotton**	United States Department of Agriculture/ Agricultur	Ascorbate peroxidase - Donor: Pea NptII* Glutathione reductase - Donor: Arab. thaliana	AP - Drought Tolerant AP - Heat Tolerant
99-048-06N	**Lettuce**	Agritope	Cyclin dependent kinase - Donor: Arab. thaliana NptII*	AP - Yield Increased
99-048-03N	**Tomato**	Agritope	Cyclin dependent kinase - Donor: Arab. thaliana NptII*	AP - Yield Increased
99-048-25N	**Persimmon**	University of California/Davis	Choline oxidase Sorbitol synthase - Donor: Apple B-glucuronidase* NptII*	AP - Cold Tolerant AP - Drought Tolerant
99-061-17N	**Cotton**	United States Department of Agriculture/ Agricultur	Ascorbate peroxidase - Donor: Pea NptII* Glutathione reductase - Donor: Arab. thaliana	AP - Drought Tolerant AP - Heat Tolerant
99-048-06N	**Lettuce**	Agritope	Cyclin dependent kinase - Donor: Arab. thaliana	AP - Yield Increased

			NptII*	
99-048-03N	**Tomato**	Agritope	Cyclin dependent kinase - Donor: Arab. thaliana NptII*	AP - Yield Increased
99-048-25N	**Persimmon**	University of California/Davis	Choline oxidase Sorbitol synthase - Donor: Apple B-glucuronidase* NptII*	AP - Cold Tolerant AP - Drought Tolerant
99-006-14N	**Tomato**	Zeneca	Trehalase - Donor: Potato NptII*	PQ - Yield Increased PQ - Dry Matter Content Increased
98-103-22N	**Creeping bentgrass**	Rutgers University	Citrate synthase - Donor: Ps. aeruginosa Hygromycin phosphotransferase*	AP - Aluminum Tolerant
98-103-23N	**Creeping bentgrass**	Rutgers University	Thiamine biosynthetic enzyme - Donor: Corn Hygromycin phosphotransferase*	AP - Drought Tolerant
98-103-30N	**Apple**	University of California	Leafy homeotic regulatory gene - Donor: Arabadopsis NptII*	AP - Flowering Time Altered
98-103-24N	**Creeping bentgrass**	Rutgers University	Hygromycin phosphotransferase* Betaine aldehyde dehydrogenase - Donor: Atriplex hortensis	AP - Salt Tolerance Increased
98-090-15N	**Wheat**	Montana State University	Phosphinothricin acetyl transferase* Aleurone 1 - Donor: Barley	AP - Drought Tolerant
98-089-84N	**Tobacco**	Southern Illinois University	Glutamate dehydrogenase NptII*	AP - Ammonium Assimilation Increased
98-083-14N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arabadopsis NptII*	AP - Oxidative Stress Tolerant
98-078-03N	**Tomato**	Zeneca	Trehalase antisense - Donor: Potato NptII*	PQ - Dry Matter Content Increased PQ - Yield Increased
98-064-20N	**Rapeseed**	Calgene	Sucrose phosphate synthase - Donor: Corn NptII*	AP - Yield Increased
98-064-25N	**Rapeseed**	Calgene	Sucrose phosphate synthase - Donor: Corn NptII*	AP - Yield Increased
98-083-14N	**Cotton**	Texas Tech University	Ascorbate peroxidase - Donor: Pea Glutathione reductase - Donor: Arabadopsis NptII*	AP - Oxidative Stress Tolerant

98-078-03N	**Tomato**	Zeneca	Trehalase antisense - Donor: Potato NptII*	PQ - Dry Matter Content Increased PQ - Yield Increased
98-064-20N	**Rapeseed**	Calgene	Sucrose phosphate synthase - Donor: Corn NptII*	AP - Yield Increased
98-064-25N	**Rapeseed**	Calgene	Sucrose phosphate synthase - Donor: Corn NptII*	AP - Yield Increased
97-266-09N	**Tobacco**	University of Hawaii/Manoa	Violaxanthin de-epoxidase antisense - Donor: Lettuce Violaxanthin de-epoxidase - Donor: Lettuca sativa Violaxanthin de-epoxidase - Donor: Tobacco NptII*	AP - Growth Rate Altered
97-241-04N	**Rapeseed**	Calgene	CBI - Donor: CBI Sucrose phosphate synthase - Donor: Corn NptII*	AP - Yield Increased
97-189-02N	**Walnut**	University of California/Davis	Leafy homeotic regulatory gene - Donor: Arabadopsis B-glucuronidase* NptII*	AP - Flowering Altered
97-157-01N	**Tomato**	University of Georgia	NptII* Phytochrome A - Donor: Oat	PQ - Seed Set Reduced PQ - Fruit Solids Increased
97-114-10N	**Tobacco**	Southern Illinois University	NptII* Glutamate dehydrogenase - Donor: E. coli B-glucuronidase*	AP - Ammonium Assimilation Increased
96-124-02N	**Tobacco**	Southern Illinois University	Glutamate dehydrogenase - Donor: E. coli NptII*	AP - Ammonium Assimilation Increased
95-340-02N	**Potato**	University of Idaho	Antifreeze protein - Donor: Synthetic Phytohemogluttinin - Donor: Bean NptII*	PQ - Cold Tolerant
95-152-01N	**Tobacco**	Southern Illinois University	Glutamine binding protein - Donor: E. coli NptII*	AP - Ammonium Assimilation Increased
95-101-14N	**Tomato**	Cornell University	Phytochrome A - Donor: Oat NptII*	AP - Shorter Stems
95-100-03N	**Corn**	University of California	B-glucuronidase* - Donor: E. coli Phosphinothricin acetyl transferase* - Donor: Strep. hygroscopicus Knotted-1 - Donor: Corn	OO - Development Altered
94-354-01N	**Cotton**	All-Tex Seed	Superoxide dismutase - Donor: Pea Superoxide dismutase - Donor: Tobacco NptII*	AP - Cold Tolerant

94-161-01N	**Corn**	University of California	Knotted-1 - Donor: Corn B-glucuronidase* Phosphinothricin acetyl transferase*	OO - Development Altered
93-287-01N	**Tobacco**	Mississippi State University	ro1c - Donor: Agro. rhizogenes NptII*	OO - Growth Rate Reduced
91-079-01R	**Tomato**	DNA Plant Tech	NptII* Protein A - Donor: Winter flounder	AP - Cold Tolerant