Targeting metabolic pathways for genetic engineering abiotic stress-tolerance in crops ☆

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ABSTRACT

Abiotic stress conditions are the major limitations in modern agriculture. Although many genes associated with plant response(s) to abiotic stresses have been indentified and used to generate stress tolerant plants, the success in producing stress-tolerant crops is limited. New technologies are providing opportunities to generate stress tolerant crops. Biotechnological approaches that emphasize the development of transgenic crops under conditions that mimic the field situation and focus on the plant reproductive stage will significantly improve the opportunities of producing stress tolerant crops. Here, we highlight recent advances and discuss the limitations that hinder the fast integration of transgenic crops into agriculture and suggest possible research directions. This article is part of a Special Issue entitled: Plant gene regulation in response to abiotic stress.

1. Introduction

The ever-increasing human population, together with the loss of agricultural land (due to urbanization, industrialization, desertification, and climatic changes) and the diminishing resources availability pose serious challenges to world agriculture. Since plant were first domesticated ~10,000 years ago and up to the present days, breeding crop plants to increase yield and feed the expanding population has been very efficient. Nevertheless, in order to feed the 9 billion people expected by 2050 (http://www.fao.org/wsfs/world-summit/en/), a significant grain yield increase of approximately 44 million metric tons per year will be needed (reviewed by [1]). These yields goals are even more challenging in light of the projected scenarios of global warming.

Water deficit, extreme temperatures (high or low) and ion imbalance (toxicity and/or deficiency) are the major abiotic stress conditions that reduce plant growth and result in significant yield losses. Although plants have evolved a wide spectrum of programs for sensing, responding and adapting to changing environment [2–8], the current understanding of the mechanisms associated with the ability of crops to maintain yield under abiotic stress are poorly understood. New advances in 'omic' technologies are providing opportunities leading to the identification of transcriptional, translational and post-translational mechanisms and signaling pathways that regulate the plant response(s)

to stress [9]. The use of model plants, such as *Arabidopsis thaliana*, *Brachypodium distachyon*, and *Medicago truncatula* provided fundamental tools for understanding the genetic and biochemical basis of abiotic stress adaptations [10,11]. Currently, numerous genes related to plant response to abiotic stress have been identified and characterized. However, a limited success in producing abiotic-stress tolerant cultivars through genetic engineering has been achieved, taking into consideration the low number of transgenic crops released to the market so far [12]. An important aspect to consider when breeding for abiotic stress tolerant crops is how to determine the success of the transgenic plants. While from a physiological perspective, survival (or recovery) is the major trait representing plant stress tolerance, from an agronomical point of view crop yield is the key determinant of successful stress-tolerant crops. Thus, while there are many studies reporting abiotic stress resistance, the majority of this work used model plants (reviewed by [13–15], tested under artificial extreme conditions (i.e. very high salinity, severe dehydration, osmotic shock, etc.) with plant recovery after a stress episode as an indication of tolerance. However, under natural field conditions, crops have to cope with multiple environmental stress which varied in time, duration and intensity (reviewed by [16]).

Currently, about 30 genetically engineered crops, occupying almost 300 million acres, are being grown in 25 countries [17]. It is expected that by 2015 more than 120 transgenic crops will be cultivated worldwide [18]. Several reviews regarding genetic engineering for improving plant tolerance to abiotic stress have been published in recent years [14,15,19,20], focusing mainly on model plants. In this review we highlight recent advances in the generation of abiotic stress-tolerant crops, and discuss the limitations that hindered the fast integration of transgenic crops into agriculture and suggest some possible research directions.

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2. Manipulating single traits: The target-gene approach

Manipulation of single genes that affect specific targets (metabolites or proteins) has been the most common strategy for improving abiotic stress tolerance in plants (reviewed by [12,14,15,20–22]. Overexpression of genes encoding enzymes associated with the accumulation of osmolytes, proteins and enzymes that function scavenging oxygen radicals (ROS), molecular chaperones and ion transporters, provided insights on the role of these genes in key physiological and biochemical processes (reviewed by [14,15,23]).

2.1. Genes associated with osmoregulation

The biosynthesis and accumulation of compatible solutes is an important adaptive mechanism that enable protection of cell turgor and restoration of water status of cells by maintaining cellular water potential as well as acting stabilizing membranes and/or scavenge ROS. These compatible solutes include amines (polyamines and glycinebetaine), amino acids (proline), sugars (trehalose, fructan), and sugar alcohols (trehalose, mannitol and galactinol) (reviewed by [24]). Overproduction of such osmoprotectans has been extensively used in several target crops in an attempt to improve tolerance to abiotic stress.

Polyamines (PAs) are low molecular weight aliphatic nitrogen compounds positively charged at physiological pH [25], which were shown to be involved in the response to abiotic stress (reviewed by [26]). The modification of PA levels by the overexpression of genes such as ornithine or arginine decarboxylases (ODC, ADC), S-adenosylmethionine (SAM) decarboxylase (SAMDC), Spermidine synthase (SPDS) in Arabidopsis [25] and tobacco (Nicotiana tabacum) [27], and in crop plants such as rice (Oryza sativa) [28–30], potato (Solanum tuberosum) [31] and eggplant (Solanum melongena) [30] was reported to result in enhanced tolerance of these species to different abiotic stresses. Glycinebetaine (GB), a fully N-methyl-substituted derivative of glycine, accumulates in the chloroplasts and other plastids of many species in response to abiotic stress and is considered the major osmolyte involved in cell membrane protection [7]. The overproduction of GB was shown to be a promising approach in developing abiotic stress tolerant plants tolerance [32]. Transgenic bread wheat (Triticum aestivum) plants overexpressing a betaine aldehyde dehydrogenase (BADH) gene, showed improved osmotic adjustment and antioxidative defense capacity which support higher photosynthetic rates leading to increased tolerance to drought and heat [33], although no yield was reported. Introducing the betA (encoding choline dehydrogenase) gene to maize (Zea mays) [34] and wheat [35] resulted in improved yield under stressful conditions in the field. Also, the expression of gene encoding choline monooxygenase (CMO, involved in GB biosynthesis) in cotton (Gossypium hirsutum) plants supported higher yield production under saline field condition [36]. However, when grown under control conditions, the transgenic plants showed reduced yield production.

Proline accumulation play adaptive role(s) in plant adaptation to osmotic stress, acts acting as a store of carbon and nitrogen and function as a molecular chaperone stabilizing the structure of proteins, (reviewed by [37]). The expression of the mothbean Δ1-pyrroline-5-carboxylate synthetase (P5CS) induced increased tolerance to stress in rice [38] and wheat [39]. On the other hand, transgenic chickpea (Cicer arietinum) expressing P5CSF129A constitutively only displayed a modest increase in transpiration efficiency, suggesting that enhanced proline had little bearing on yield in chickpea [40]. Soybean plants expressing Δ^1-pyrroline-5-carboxylate reductase (P5CR) under the control of an inducible heat shock promoter were found to accumulate higher amounts of proline without deleterious effects in growth being able to retain higher relative water content (RWC) and higher glucose and fructose levels than the antisense and control plants, confering drought stress tolerance [41]. The contrasting results obtained for manipulating P5CS

gene in various crops could result from either different metabolomic pathways involved in stress tolerance in various species, epigenetics, and or the experimental design.

Overproducing mannitol in wheat, which does not synthesize mannitol normally, by constitutively expressing the mannitol-1-phosphate dehydrogenase (mtlD) gene resulted in improved tolerance to drought and salinity in terms of growth. However, under control conditions, growth was accompanied with sterility [42]. The ameliorative effect of mannitol was likely to be exerted through the scavenging of hydroxyl radicals and stabilization of macromolecular structures ([42] and references therein). Trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside) which is specially accumulated in desiccation-tolerant "resurrection plants" [43], was engineered in plants either by regulation of trehalase activity [44] or by expression of trehalose synthesis-related genes [45]. Overexpression of two Escherichia coli trehalose biosynthetic genes (otsA and otsB) was shown to improve tolerance to abiotic stresses in rice [46] and alfalfa (Medicago sativa) [47]. In general, stunted growth of the transgenic plants was avoided when an ABA-inducible (rd29A) promoter was used [45,47]. The results reported above illustrate the potential of manipulating osmolyte accumulation to genetically engineer abiotic stress tolerant crop plants.

2.2. Detoxification of reactive oxygen species

Abiotic stresses induce the generation of reactive oxygen species (ROS) such as $^1O_2$, H_2O_2, $O_2^{\bullet-}$ and $HO\cdot$ [48]. ROS are toxic molecules that cause oxidative damage to proteins, DNA and lipids [49]. Enzymatic scavenging of ROS involves proteins of the aldehyde dehydrogenases (ALDHs) family, which catalyzes the conversion of aldehydes to the corresponding acids playing an important role in detoxification of acetaldehydes [50]. Overexpression of Mn-superoxide dismutase (Mn SOD3.1), that mediates the conversion of O_2^- to H_2O_2, in alfalfa [51], wheat [52] and potato [53] resulted in higher tolerance to abiotic stress and improved yields under field conditions. Ascorbate peroxidases (APX) and catalases (CAT) are two important enzymes that participate in ROS detoxification. Expression of cAPX gene in tomato (Solanum lycopersicum) improved tolerance to exposure to direct sunlight, under field conditions [54] and expression of the katE gene in rice, resulted in improved growth and yield under salt stress [55]. The expression of a combination of antioxidant enzymes was shown to be a promising strategy to enhance abiotic stress tolerance. Transgenic rice plants, constitutively co-expressing Glutathione S-transferase (GST) and CAT genes showed enhanced tolerance to salinity and oxidative stresses at the vegetative stage [56]. In tobacco, co-expression of three antioxidant enzymes, copper zinc superoxide dismutase (CuZnSOD), APX, and dehydroascorbate reductase (DHAR) resulted in a higher tolerance to salt stress [57]. While many studies have demonstrated that increasing the antioxidant capacity of a plant improves abiotic stress tolerance, testing how these transgenic plants perform under field conditions is needed to confirm the beneficial effect on yield.

2.3. Late embryogenesis abundant proteins

The manipulation the expression of genes encoding for chaperones (CSPs), heat-shock proteins (HSP) and late embryogenesis abundant (LEA) proteins have been widely used for improving stress tolerance in plants (reviewed by [15]). LEA proteins are low molecular weight proteins that play crucial roles in cellular dehydration tolerance preventing protein aggregation during desiccation or water-stress, having antioxidant capacity together with a possible role as chaperones [58–60]. Overexpression of OsLEA3-1 in rice, resulted in improved yields under drought stress, without yield penalties under control conditions [61]. The barley (Hordeum vulgare) LEA protein HVA1, was shown to improve yields under drought stress in transgenic wheat [62] and rice [63]. Dehydrins are a subfamily of group 2 LEA proteins [64] that accumulate in vegetative tissues subjected to drought, salinity and cold

stress. Strawberry (*Fragaria × anassassa*) overexpressing a wheat dehydrin *WCOR410* gene showed improved leaf freezing tolerance [65]. Recently, it has been demonstrated that the dehydrin gene *Lti30* is involved in cold stress tolerance by interacting electrostatically with vesicles of both zwitterionic (phosphatidyl choline) and negatively charged phospholipids (phosphatidyl glycerol, phosphatidyl serine, and phosphatidic acid) [66]. This strategy still needs to be tested in crops under field conditions. The expression of two members of a family of bacterial RNA chaperones, *E. coli CspA* and *B. subtilus CspB*, resulted in enhance tolerance to abiotic stress, by maintaining growth, photosynthesis and development in rice, maize and *Arabidopsis* [67]. Multiple locations and years field trials with the transgenic maize expressing *CspA* and *CspB* showed improved yields (11–21%) under water-stress conditions when tested in multiple field locations. Importantly, the improvements in water-limited field trials were not associated with a yield penalty in high-yielding environments [67]. Overexpressing the rice small heat-shock protein gene, *sHSP17.7*, which shown to act as molecular chaperones resulted in improved drought and osmotic stress tolerance (as seedling survival rate) [68]. While LEA, CSPs, and HSP proteins have been repeatedly shown to be involved in abiotic stress response (reviewed by [59]), only limited experiments have used this strategy for engineering abiotic stress tolerant crops.

2.4. Regulation of water and ion homeostasis

The ability to maintain water content under stress conditions is critical for plant survival. Aquaporins are intrinsic membrane proteins that mediate the transport of water, small neutral solutes and CO_2 [69,70]. These membrane proteins implicated in water diffusion, are regulated in response to environmental cues and particularly in ABA dependent stomatal conductance pathway [71]. The use of aquaporins for developing transgenic plants with improved tolerance to abiotic stress resulted in contrasting results. *Arabidopsis* plants expressing the wild soybean (*Glycine soja*) tonoplast intrinsic protein (TIP), *GsTIP2;1*, showed more sensitivity to salt and dehydration presumably due to enhanced water loss of the transgenic plants [72]. Transgenic tobacco plants constitutively expressing the *Arabidopsis* plasma membrane aquaporin (PIP), *PIP1b*, wilted rapidly during water-stress [73]. Similarly, transgenic rice plants constitutively overexpressing a barley *HvPIP2;1*, showed more sensitivity (reduction growth rate) to salinity stress [74]. In contrast, heterologous overexpression of rice *OsPIP-1* and *OsPIP2-2* in *Arabidopsis* resulted in improved salinity and dehydration tolerance [75]. Overexpression of wheat nodulin 26-like intrinsic proteins (NIP) gene, *TaNIP*, in *Arabidopsis* enhanced plants tolerance to abiotic stresses. Recently, a tobacco gene encoding aquaporin (*NtAQP1*) was shown to provide protection against salinity stress in transgenic tomatoes [76]. *NtAQP1* plays a key role in increasing mesophyll CO_2 permeability (supporting increased photosynthetic rate), increasing stomata aperture and preventing hydraulic failure under high xylem tensions. The higher transpiration rate and higher CO_2 assimilation rate of the transgenic plants resulted in significant improved productivity under control and salt stress [76]. Tomato plants constitutively overexpressing the TIP aquaporin gene *SlTIP2;2* showed increased cell water permeability and whole-plant transpiration, which resulted in improved salt and drought tolerance under field conditions [77].

Under saline conditions, Na^+ and Cl^- are the predominant toxic ions for cell metabolism affecting plant growth and development. Maintaining a high cytosolic K^+/Na^+ ratio is essential for plant salt tolerance [78]. Ion transporters can limit Na^+ accumulation in the cytosol by restricting Na^+ uptake, by accumulating Na^+ in the vacuole, and/or by extrusion of Na^+ out of the cells. Sodium entry into the root cells is mediated by uniporter or ion channel type transporters, like HKT, LCT1, and NSCC (reviewed by [79]). Reduction in Na^+ uptake by antisense suppression of *TaHKT2;1* gene in wheat resulted in lower net Na^+ uptake of transgenic roots under salinity stress [80]. However, this strategy was not tested in the field yet. Sodium efflux from the roots is an active

process, which is presumed to be mediated by plasma membrane Na^+/H^+ antiporters. The Na^+/H^+ antiporter salt overly sensitive 1 (SOS1), is the only Na^+ efflux protein on the plasma membrane characterized so far in plants involved in Na^+ extrusion and long-distance Na^+ transport [81]. Transgenic rice plants constitutively expressing the yeast (*Schizosaccharomyces pombe*) Na^+/H^+ antiporter *sodium2* (*SOD2*) gene, showed higher accumulation of K^+, Ca^{2+}, Mg^{2+} and less Na^+ in the shoots as compared to wild type plants [82]. The transgenic rice plants were able to maintain higher photosynthetic levels and root proton transport capacity, whereas ROS generation was reduced. Accumulation of Na^+ ions into vacuoles through the operation of vacuolar Na^+/H^+ antiporters is an efficient strategy to avert the deleterious effect of Na^+ in the cytosol [83,84]. Overexpression of an *Arabidopsis* vacuolar Na^+/H^+ antiporter, *AtNHX1*, resulted in improved salt tolerance in canola [85], tomato [22], cotton [86], wheat [87], beet (*Beta vulgaris*) [88] and tall fescue (*Festuca arundinacea*) [89]. Likewise, expression of the rice ortolog, *OsNHX1*, in rice [90] and maize [91] showed improved salt stress tolerance. Moreover, under field conditions, the transgenic maize plants produced higher grain yields than the wild-type plants. Transformation of another Na^+/H^+ antiporter family member, *AtNHX3*, in sugar beet resulted in increased salt accumulation in leaves, but not in the storage roots, with enhanced constituent soluble sugar contents under salt stress conditions [92]. Recently, overexpression of the *Arabidopsis* intracellular Na^+/H^+ antiporter *AtNHX5* [93] resulted in enhanced salt and drought tolerance in rice seedlings [94] and paper mulberry (*Broussonetia papyrifera* L. Vent) [95].

3. Targeting pathways: The manipulation of regulatory genes

The approach of manipulating single gene encoding specific metabolic pathway to improve tolerance to abiotic stress in crops had very limited success. The multiple pathways involved in plant adaptation to stress and the complexity of interactions can explain to some extend why such an approach will not work in the field. Moreover, plants always tend to restore the metabolic homeostasis, and therefore can play in contrast to the manipulated enzyme. On the other hand, targeting key regulation genes which affect multiple pathways affecting metabolic fluxes could help to restore plant metabolic homeostasis during stress episodes, increasing the probability of success. Transcription factors (TFs) are involved in almost all biological processes, and therefore likely to be good target candidates for the generation of stress-tolerant crops [96]. Different families of TF such as ERF/AP2, HSF, bZIP, MYB, MYC, NFY, NAC, WRKY, Cys_2His_2, MADS-box and zinc-finger have been shown to regulate the expression of stress-responsive genes [11,96]. Nuclear factor Y (NF-Y) complex is comprised of three subunits; NF-YA (HAP2), NF-YB (HAP3), and NF-YC (HAP5) [97], and was found to confer tolerance to abiotic stress in *Arabidopsis* [98]. Transgenic maize constitutively expressing *ZmNF-YB2* showed enhanced tolerance to severe drought stress in field trials [98]. Under water-limiting conditions, transgenic plants displayed improved grain yield, as well as reduced wilting, lower leaf temperature, etc. The NAC [*NAM* (No Apical Meristem), *ATAF1-2*, and *CUC2* (Cup-Shaped Cotyledon)] TF have been reported to be associated with abiotic stress. Transgenic rice overexpressing *SNAC1* (*STRESS-RESPONSIVE NAC 1*) showed increased yield when grown under drought stress field conditions, throughout the control of stomata movement and maintenance of photosynthetic activity [99–101]. Likewise, the overexpression of two NAC genes, *OsNAC5* and *OsNAC6*, resulted in stress tolerant rice via the up-regulation of the expression of stress-inducible genes such as *OsLEA3* [99]. Recently, expression of *OsNAC10* under control of a root-specific promoter (*RCc3*) yielded more grain in the field under drought conditions [102]. The yield advantage of P_{RCc3}::*OsNAC10* transgenic rice plants was associated with a larger root diameter [102].

Dehydration-responsive element (DRE)/C-repeat (CRT) proteins have been indentified to play important roles in drought, cold and salinity response [103]. Overexpression of *CBF1/DREB1B* genes resulted

in improved tolerance to drought, salinity and temperature stress in model plants [104–107] and in crop plants such as rice, wheat and canola [108,109]. At the same time, the transgenic plants showed negative phenological abnormalities such as severe growth retardation under control condition [110]. This problem was reduced when using more specific promoter, such as the ABA-inducible (*rd29a*) promoter [19]. The DRE-binding (*DREB*) and ethylene responsive element binding factors (*ERF*) subfamilies that belong to the large family of TFs APETALA2/ethylene-responsive (*AP2/EREBP*), mediate plant signal transduction pathways in response to environmental cues. The overexpression of HARDY (*HRD*), encoding a AP2/ERE-like TF, in rice resulted in reduced transpiration and increase water use efficiency (WUE) under control and drought conditions [111]. Although WUE is a critical parameter associated with improved stress tolerance of plants, it does not necessarily reflect higher productivity under stress conditions (reviewed by [112]) and yield parameters have to be determined.

4. Challenging hormone homeostasis

Phytohormones regulate every aspect of plant growth, development and the responses of plants to environmental cues [113–127]. The hormonal response machinery rapidly alters gene expression by inducing, preventing or controlling the degradation of regulators as TFs via the ubiquitin–proteasome pathway [128]. One of the primary plant responses to stress is the accumulation of ABA which results in stomatal closure and reduced water loss via transpiration [129,130]. While a large number of genes associated with abscisic acid (ABA) metabolic pathways have been indentified in *Arabidopsis* using loss and gain of function (reviewed by [120,131]), only a few genes involved in ABA metabolism has been successfully manipulated in crops to attain drought tolerance. Transgenic rice plants overexpressing *LOS5/ABA3*, a key enzyme in the last step of ABA biosynthesis, showed improved yield in the field under drought stress [132]. In tomatoes, overexpression of *LeNCED1* (a drought-inducible gene encoding a rate-limiting enzyme in ABA biosynthesis) resulted in increased ABA accumulation and improved drought tolerance [133]. However, under control conditions the transgenic tomato plants showed negative physiological and morphological changes associated with the constant increase of ABA level, which resulted in the reduction of assimilation rates. *ERA1* encodes the β–subunit of farnesyl transferase, an enzyme associated with ABA signaling. Transgenic canola carrying *era1* antisense (driven by the drought-inducible *rd29A* promoter displayed enhanced yield under mild drought conditions in the field [134]. These results further highlight the need of specific promoters to control gene expression and to avoid negative effects [133]. Recently, overexpression of a Harpin-encoding (*hrf1*) gene in rice was shown to improve drought tolerance through ABA signaling promoting stomatal closure increasing the levels of free proline [135].

Cytokinin (CK) has been found to be associated with plant responses to various abiotic stresses (reviewed by [136,137]). CK could promote survival under drought stress, inhibiting leaf senescence and increasing levels of proline [138]. Modification of endogenous CK levels was shown to be an effective strategy in delaying senescence processes [139]. *IPT*, a gene encoding isopentenyltransferase, an enzyme mediating the rate-limiting step in CK biosynthesis, has been overexpressed in several plant species [140]. Transgenic plants varied depending on the type of promoter used to drive *IPT* expression [141]. Expression of the *IPT* gene under the control of *SARK* (senescence associated receptor kinase), a maturation- and stress-induced promoter, in both tobacco and rice resulted in increased drought tolerance, without the negative effects of high CK content on plant phenology [142–145]. The transgenic plants displayed enhanced drought tolerance and superior yields compared with wild type plants [142]. Transgenic Cassava (*Manihot esculenta* Crantz), expressing *IPT* under control of a senescence induced promoter, *SAG12*, was tested for drought tolerance under field conditions displaying higher tolerance to the stress due to the inhibition of

stress-induced leaf abscission and fast recovery from stress [146]. Tomato plants grafted on rootstocks constitutively expressing *IPT* resulted in a decrease of root biomass under control conditions while under salinity-stress conditions the transgenic plants yielded 30% more than the wild type plants [147].

5. Targeting pathways: Expressing genes in tandem

Under natural field conditions plants have to cope with different stress combinations at different developmental stages and for varying duration. Tolerance to abiotic stress is a consequence of genetic and environmental interactions through a complex network that implies physiological, molecular and biochemical responses. Modifying the expression of different components simultaneously has the potential to generate responses apt to the complexity of a combination of stresses. There are only few examples where the simultaneous co-expression of different components of the same pathway has been tried. Increase in biosynthesis of proline was achieved by co-expression of *E. coli P5C* biosynthetic enzymes gamma-glutamyl kinase 74 (*GK74*) and gamma-glutamylphosphate reductase (*GPR*) and the antisense transcription of proline dehydrogenase (*ProDH*) in *Arabidopsis* and tobacco [148]. The transgenic plants displayed improved tolerance to heat stress associated with the accumulation of cell wall proline-rich proteins [148]. Simultaneous co-expression of dehydroascorbate reductase (DHAR), glutathione reductase (GR) or glutathione-S-transferase (GST) and glutathione reductase (GR) in tobacco plants also resulted in the increased tolerance of the transgenic plants to a variety of abiotic stresses [149]. In tobacco seeds, higher antioxidant enzymes activity driven by the simultaneous overexpression of the *CuZnSOD* and *APX* genes in plastids, allowed the increase of germination rates and longevity of long-term stored seeds under combined stress conditions [150], demonstrating the enormous potential of simultaneous gene expression in plant engineering.

6. Epigenetic and post-transcriptional control

Epigenetic processes such as DNA methylation, histone modifications, generation of small RNAs (sRNA) molecules and transposable element activity, play essential roles in modulating gene activity in response to environmental stimuli [151–153]. While most mechanisms involved in epigenetic and its heritance have not yet indentified, they play a major role in gene silencing on one hand and as a target for manipulation on the other. Abiotic stress can induce changes in gene expression through hypomethylation or hypermethylation of DNA which are related with stress tolerance. The stress-induced-specific CpHpG-hypermethylation in the halophyte *Mesembryanthemum crystallinum* L. induced the switch in photosynthesis mode from C_3 to CAM, contributing to the adaptation to salt stress [154]. In wheat, the use of the methylation inhibitor 5-azacytidine resulted in the increased tolerance to salt stress at the seedling stage [155]. Decrease levels of histone acetylation levels (antisense) in tomato resulted in higher photosynthetic rates under water-stress [156]. The control of methylation and histone patterns is emerging as a potential tool for improving tolerance to abiotic stress in crops, however, little is known about how to control the effect of post transcriptional manipulation.

Small non-coding RNAs, including small RNAs (sRNAs), short interfering RNAs (siRNAs) and micro RNAs (miRNA), have been to be important regulators of protein-coding gene expression [157,158], controlling mRNA stability and translation, or targeting epigenetic modifications. Abiotic stress can induce both the over- or under-expression of specific sRNAs that are involved in pathways that contribute to re-program complex processes of metabolism and physiology. Several reports have recently indicated the possible use of these sRNAs as targets for the genetic manipulation of crops. The overexpression of *miR398* in *Arabidopsis*, which targets two closely related Cu/Zn superoxide dismutases (cytosolic *CSD1* and chloroplastic *CSD2*) resulted in increased tolerance to oxidative stress [159].

Transgenic tomatoes expressing *Sly-miR169c* displayed decreased stomata opening, a decrease in leaf water loss and enhanced drought tolerance [160]. Transgenic rice constitutively expressing *osa-MIR396c* showed increased sensitivity to salt stress [161]. The identification and characterization of the role(s) of sRNAs in the regulation of gene expression (reviewed by [162]), together with the development of artificial miRNA methodologies [163] open new avenues for the generation of transgenic stress tolerant crops.

7. Modifying function: Engineering C_4 photosynthetic pathway into C_3 crops

Abiotic stress is the major factor limiting photosynthetic activity, resulting in growth and yield reduction. The photosynthesis machinery also affects metabolic processes such as carbon and nitrogen partitioning [164–166] and oxidative stress regulation [167]. The projected effects of climate change in rising ambient temperatures and CO_2 concentrations will have influence plant CO_2 assimilation (and yield), and photorespiration. The ability of the C_4 photosynthetic pathway to suppress ribulose 1,5-bisphosphate (RuBP) oxygenation and photorespiration represents the most efficient form of photosynthesis on Earth [168]. In recent years, efforts have been given to engineer C_4 photosynthesis into C_3 crops [169,170]. The expression of genes encoding enzymes such as phosphoenolpyruvate carboxylase (PEPC), the chloroplastic pyruvate orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME) into rice [171–174], tobacco [175] and potato [176] improved photosynthetic rate and yield. Although considerable efforts have been made, the overexpression of either single or multiple C_4-enzyme related genes in C_3 plants have resulted in contradictory results. [170,177,178].

Research efforts are also focused on obtaining Kranz anatomy [169], especially in rice which have an intermediate anatomical characteristics between C_3 and C_4 plants [179]. While most genes controlling bundle density in C_4 plants are still unknown, it has been postulated that about 20 genes will be required (reviewed by [180]). Thus, in order to obtain C_4 crops, new transformation methods together with additional efforts to better understand the function of C_4 enzymes in a proper leaf anatomy [178] are needed. Thus, in order to obtain C_4 crops, new transformation methods are needed. Another important aspect that has to be addressed is source/sink relationships. From an evolutionary perspective C_3 plants have modified their sink size proportionally to the source size (i.e. photosynthesis organs). Thus, more efficient carbon fixation via C_4 pathway in the transformed plants would require to adapt the sinks to attain efficient harvest index [181].

8. When and how much to express: The key role of promoters

Most of the genes engineered into crops to improve abiotic stress tolerance were driven by constitutive promoters. In general, the most common promoters used for the manipulation of gene expression are the Cauliflower mosaic virus *35S* (*CaMV35S*; [182]), ubiquitin (*UBI1*; [183]) or actin [184]. Although these promoters have been effective in the production of transgenic plants with enhanced stress tolerance, the constitutive expression of candidate genes is not always desirable because of negative (pleiotropic) effects on growth and development under control conditions. This appears to be very relevant with the manipulation of key regulatory genes such as transcription factors or enzymes mediating plant hormone synthesis (reviewed by [113]). A solution to this problem is the use of stress-inducible promoters that allow transgene expression during the stress episode. As an example, the constitutive expression (*35S*) of the *Arabidopsis CBF1* in transgenic tomato plants resulted in improved tolerance to chilling, drought and salt stress, whereas under normal conditions the transgenic plants showed a dwarf phenotype and reduction in fruit set [185]. In contrast, when the same gene was driven by an inducible promoter (barley *HAV22*) the transgenic tomato plants exhibited enhanced tolerance to

the applied stresses with no effect in growth and yield under control conditions. The constitutive expression of *IPT* or *knotted 1* (*kn1*, a homeobox gene) under the control of *35S* in tobacco plants resulted in leaf and plant size reduction, altered leaf shape, loss of apical dominance, delay in senescence, and formation of ectopic meristems [186]. In contrast, the use of stress-induced promoters (*SARK*) to drive *IPT* expression did not altered plant phenology and resulted in enhanced drought tolerance of the transgenic plants [142–145]. The use of strong constitutive promoters to control the expression of transgenes could accelerate the process of RNA silencing [187] that can occur at the transcriptional (TGS) and post-transcriptional (PTGS) levels (reviewed by [188]). Besides, gene expression in specific cell types has resulted in the increase of salt tolerance when enhancer trap system was used to drive root stele specific expression of *HKT1*, but not when driven constitutively using 35S [189]. These results support the use of the conditional expression of the gene of interest as a useful strategy to control gene expression, without the negative effects on growth and development, and possibly reducing epigenetic effects of the transgene.

9. Conclusions and perspectives

New technologies are providing opportunities to generate transgenic crops able to maintain high yields under stressful and changing environments. Many genes associated with plant response(s) to abiotic stresses have been identified and used to generate stress tolerant plants. Most of these studies were conducted under laboratory conditions applying artificial stress conditions, using model plants and focusing on recovery from a stress episode as the main trait. However, crops grown in the field face heterogeneous conditions and are exposed to the simultaneous occurrence of different stresses (reviewed by [16]). Thus, more emphasis should be placed on the development of transgenic crops under conditions that mimic the field situation (i.e. combination of environmental stresses) and focus on the plant reproductive stage (during flowering and seed/fruit/grain maturation), the most critical stage determining crop yield. From a biotechnological stand, the interaction of transgene×environment can have significant effects that will depend on the conditions (i.e. greenhouse *versus* field, vegetative stage *versus* reproductive stage, etc.) at which plants are phenotyped. For example, Zeller et al. [190] showed, using transgenic wheat expressing the powdery mildew resistance gene, *Pm3*, as model, that while the transgenic lines displayed the desired phenotype across a range of environments in a greenhouse experiment, some of these effects were reversed when the transgenic lines were grown in the field.

References

[1] M. Tester, P. Langridge, Breeding technologies to increase crop production in a changing world, Science 327 (2010) 818–822.
[2] D. Bartels, R. Sunkar, Drought and salt tolerance in plants, Crit. Rev. Plant Sci. 24 (2005) 23–58.
[3] E.A. Bray, Molecular responses to water deficit, Plant Physiol. 103 (1993) 1035–1040.
[4] E.A. Bray, Plant responses to water deficit, Trends Plant Sci. 2 (1997) 48–54.
[5] M.M. Chaves, J.P. Maroco, J.S. Pereira, Understanding plant responses to drought — from genes to the whole plant, Funct. Plant Biol. 30 (2003) 239–264.
[6] R. Munns, Comparative physiology of salt and water stress, Funct. Plant Biol. 25 (2002) 239–250.
[7] R. Munns, M. Tester, Mechanisms of salinity tolerance, Annu. Rev. Plant Biol. 59 (2008) 651–681.

[8] J.R. Witcombe, P.A. Hollington, C.J. Howarth, S. Reader, K.A. Steele, Breeding for abiotic stresses for sustainable agriculture, Philos. Trans. R. Soc. B 363 (2008) 703–716.

[9] I. Ahuja, R.C.H. de Vos, A.M. Bones, R.D. Hall, Plant molecular stress responses face climate change, Trends Plant Sci. 15 (2010) 664–674.

[10] H.J. Bohnert, Q. Gong, P. Li, S. Ma, Unraveling abiotic stress tolerance mechanisms — getting genomics going, Curr. Opin. Plant Biol. 9 (2006) 180–188.

[11] T. Hirayama, K. Shinozaki, Research on plant abiotic stress responses in the post-genome era: past, present and future, Plant J. 61 (2010) 1041–1052.

[12] Z. Peleg, M.P. Apse, E. Blumwald, Engineering salinity and water-stress tolerance in crop plants: getting closer to the field, Adv. Bot. Res. 57 (2011) 405–443.

[13] M. Ashraf, N.A. Akram, Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison, Biotechnol. Adv. 27 (2009) 744–752.

[14] J.M. Pardo, Biotechnology of water and salinity stress tolerance, Curr. Opin. Plant Biol. 21 (2010) 185–196.

[15] T. Umezawa, M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki, K. Shinozaki, Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future, Curr. Opin. Plant Biol. 17 (2006) 113–122.

[16] R. Mittler, E. Blumwald, Genetic engineering for modern agriculture: challenges and perspectives, Annu. Rev. Plant Biol. 61 (2010) 443–462.

[17] J. Clive, Global Status of Commercialized Biotech/GM Crops, The International Service for the Acquisition of Agri-biotech Applications (ISAAA) Brief No.41, Ithaca, NY, 2009.

[18] A.J. Stein, E. Rodriguez-Cerezo, The global pipeline of new GM crops: implications of asynchronous approval for international trade, in: J.R.C.E. Commission (Ed.) JRC Scientific and Technical Reports, Institute for Prospective Technological Studies, Joint Research Centre, Institute for Prospective Technological Studies, Seville, Spain, 2009.

[19] S. Yang, B. Vanderbeld, J. Wan, Y. Huang, Narrowing down the targets: towards successful genetic engineering of drought-tolerant crops, Mol. Plant 3 (2010) 469–490.

[20] E. Cominelli, C. Tonelli, Transgenic crops coping with water scarcity, N. Biotechnol. 27 (2010) 473–477.

[21] K. Nakashima, Y. Ito, K. Yamaguchi-Shinozaki, Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses, Plant Physiol. 149 (2009) 88–95.

[22] H.-X. Zhang, E. Blumwald, Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit, Nat. Biotechnol. 19 (2001) 765–768.

[23] B. Vinocur, A. Altman, Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations, Curr. Opin. Plant Biol. 16 (2005) 123–132.

[24] D. Rontein, G. Basset, A.D. Hanson, Metab. Eng. of osmoprotectant accumulation in plants, Metab. Eng. 4 (2002) 49–56.

[25] R. Alcázar, F. Marco, J. Cuevas, M. Patron, A. Ferrando, P. Carrasco, A. Tiburcio, T. Altabella, Involvement of polyamines in plant response to abiotic stress, Biotechnol. Lett. 28 (2006) 1867–1876.

[26] S.S. Hussain, M. Ali, M. Ahmad, K.H.M. Siddique, Polyamines: natural and engineered abiotic and biotic stress tolerance in plants, Biotechnol. Adv. 29 (2008) 300–311.

[27] S. Wi, W. Kim, K. Park, Overexpression of carnation S-adenosylmethionine decarboxylase gene generates a broad-spectrum tolerance to abiotic stresses in transgenic tobacco plants, Plant Cell Rep. 25 (2006) 1111–1121.

[28] T. Capell, L. Bassie, P. Christou, Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 9909–9914.

[29] M. Roy, R. Wu, Overexpression of S-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloride-stress tolerance, Plant Sci. 163 (2002) 987–992.

[30] V.R. Prabhavathi, M.V. Rajam, Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance, Plant Biotechnol. 24 (2007) 273–282.

[31] Y. Kasukabe, L. He, Y. Watakabe, M. Otani, T. Shimada, S. Tachibana, Improvement of environmental stress tolerance of sweet potato by introduction of genes for spermidine synthase, Plant Biotechnol. 23 (2006) 75–83.

[32] S. Lv, A. Yang, K. Zhang, L. Wang, J. Zhang, Increase of glycinebetaine synthesis improves drought tolerance in cotton, Mol. Breed. 20 (2007) 233–248.

[33] G.-P. Wang, Z. Hui, F. Li, M.-R. Zhao, J. Zhang, W. Wang, Improvement of heat and drought photosynthetic tolerance in wheat by overaccumulation of glycinebetaine, Plant Biotechnol. Rep. 4 (2010) 213–222.

[34] R. Quan, M. Shang, H. Zhang, Y. Zhao, J. Zhang, Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize, Plant Biotechnol. J. 2 (2004) 477–486.

[35] C. He, H. Yang, W. Zhang, Q. Gao, J. Zhang, Improved salt tolerance of transgenic wheat by introducing betA gene for glycine betaine synthesis, Plant Cell Tissue Org. 101 (2010) 65–78.

[36] T.H.H. Chen, N. Murata, Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications, Funct. Plant Biol. 34 (2011) 1–20.

[37] N. Verbruggen, C. Hermans, Proline accumulation in plants: a review, Amino Acids 35 (2008) 753–759.

[38] J. Su, R. Wu, Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis, Plant Sci. 166 (2004) 941–948.

[39] E.C.G. Vendruscolo, I. Schuster, M. Pileggi, C.A. Scapim, H.B.C. Molinari, C.J. Marur, L.G.E. Vieira, Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat, J. Plant Physiol. 164 (2007) 1367–1376.

[40] P. Bhatnagar-Mathur, V. Vadez, M. Jyostna Devi, M. Lavanya, G. Vani, K. Sharma, Genetic engineering of chickpea (Cicer arietinum L.) with the P5CSF129A gene for osmoregulation with implications on drought tolerance, Mol. Breed. 23 (2009) 591–606.

[41] J.A. de Ronde, R.N. Laurie, T. Caetano, M.M. Greyling, I. Kerepesi, Comparative study between transgenic and non-transgenic soybean lines proved transgenic lines to be more drought tolerant, Euphytica 138 (2004) 123–132.

[42] T. Abebe, A.C. Guenzi, B. Martin, J.C. Cushman, Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity, Plant Physiol. 131 (2003) 1748–1755.

[43] R.P. Adams, E. Kendall, K.K. Kartha, Comparison of free sugars in growing and desiccated plants of Selaginella lepidophylla, Biochem. Syst. Ecol. 18 (1990) 107–110.

[44] O. Goddijn, T.C. Verwoerd, E. Voogd, R. Krutwagen, P. de Graff, J. Poels, K. van Dun, A.S. Ponstein, B. Damm, J. Pen, Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants, Plant Physiol. 113 (1997) 181–190.

[45] I.-C. Jang, S.-J. Oh, J.-S. Seo, W.-B. Choi, S.I. Song, C.H. Kim, Y.S. Kim, H.-S. Seo, Y.D. Choi, B.H. Nahm, J.-K. Kim, Expression of a bifunctional fusion of the Escherichia coli genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth, Plant Physiol. 131 (2003) 516–524.

[46] A.K. Garg, J.-K. Kim, T.G. Owens, A.P. Ranwala, Y.D. Choi, L.V. Kochian, R.J. Wu, Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 15898–15903.

[47] R. Suarez, C. Calderon, G. Iturriaga, Enhanced tolerance to multiple abiotic stresses in transgenic alfalfa accumulating trehalose, Crop. Sci. 49 (2009) 1791–1799.

[48] N. Suzuki, S. Koussevitzky, R. Mittler, G. Miller, ROS and redox signaling in the response of plants to abiotic stress. Plant Cell Environ. (in press) doi:10.1111/j.1365-3040.2011.02336.x.

[49] G. Miller, N. Suzuki, S. Ciftci-Yilmaz, R. Mittler, Reactive oxygen species homeostasis and signalling during drought and salinity stresses, Funct. Plant Biol. 33 (2010) 453–467.

[50] M. Nakazono, H. Tsuji, Y. Li, D. Saisho, S.-i. Arimura, N. Tsutsumi, A. Hirai, Expression of a gene encoding mitochondrial aldehyde dehydrogenase in rice increases under submerged conditions, Plant Physiol. 124 (2000) 587–598.

[51] B.D. McKersie, S.R. Bowley, E. Harjanto, O. Leprince, Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase, Plant Physiol. 111 (1996) 1177–1181.

[52] L. Gusta, N. Benning, G. Wu, X. Luo, X. Liu, M. Gusta, A. McHughen, Superoxide dismutase: an all-purpose gene for agri-biotechnology, Mol. Breed. 24 (2009) 103–115.

[53] D. Waterer, N. Benning, G. Wu, X. Luo, X. Liu, M. Gusta, A. McHughen, L. Gusta, Evaluation of abiotic stress tolerance of genetically modified potatoes (Solanum tuberosum cv. Desiree), Mol. Breed. 25 (2010) 527–540.

[54] Y. Wang, M. Wisniewski, R. Meilan, M. Cui, L. Fuchigami, Transgenic tomato (Lycopersicon esculentum) overexpressing cAPX exhibits enhanced tolerance to UV-B and heat stress, J. Appl. Horticulture 8 (2006) 87–90.

[55] K. Nagamiya, T. Motohashi, T. Nakao, S. Prodhan, E. Hattori, S. Hirose, K. Ozawa, Y. Ohkawa, T. Takabe, T. Takabe, A. Komamine, Enhancement of salt tolerance in transgenic rice expressing an Escherichia coli catalase gene, kat E, Plant Biotechnol. Rep. 1 (2007) 49–55.

[56] F. Zhao, H. Zhang, Salt and paraquat stress tolerance results from co-expression of the Suaeda salsa glutathione S-transferase and catalase in transgenic rice, Plant Cell Tissue Org. 86 (2006) 349–358.

[57] Y.-P. Lee, S.-H. Kim, J.-W. Bang, H.-S. Lee, S.-S. Kwak, S.-Y. Kwon, Enhanced tolerance to oxidative stress in transgenic tobacco plants expressing three antioxidant enzymes in chloroplasts, Plant Cell Rep. 26 (2007) 591–598.

[58] K. Goyal, L.J. Walton, A. Tunnacliffe, LEA proteins prevent protein aggregation due to water stress, Biochem. J. 388 (2005) 151–157.

[59] S.C. Hand, M.A. Menze, M. Toner, L. Boswell, D. Moore, LEA proteins during water stress: not just for plants anymore, Annu. Rev. Physiol. 73 (2011) 115–134.

[60] D. Kovacs, E. Kalmar, Z. Torok, P. Tompa, Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins, Plant Physiol. 147 (2008) 381–390.

[61] B. Xiao, Y. Huang, N. Tang, L. Xiong, Over-expression of a LEA gene in rice improves drought resistance under the field conditions, Theor. Appl. Genet. 115 (2007) 35–46.

[62] A. Bahieldin, H.T. Mahfouz, H.F. Eissa, O.M. Saleh, A.M. Ramadan, I.A. Ahmed, W.E. Dyer, H.A. El-Itriby, M.A. Madkour, Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance, Physiol. Plant. 123 (2005) 421–427.

[63] D. Xu, X. Duan, B. Wang, B. Hong, T. Ho, R. Wu, 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 (1996) 249–257.

[64] M. Battaglia, Y. Olvera-Carrillo, A. Garciarrubio, F. Campos, A.A. Covarrubias, The enigmatic LEA proteins and other hydrophilins, Plant Physiol. 148 (2008) 6–24.

[65] M. Houde, S. Dallaire, D. N'Dong, F. Sarhan, Overexpression of the acidic dehydrin WCOR410 improves freezing tolerance in transgenic strawberry leaves, Plant Biotechnol. J. 2 (2004) 381–387.

[66] S.K. Eriksson, M. Kutzer, J. Procek, G. Gröbner, P. Harryson, Tunable membrane binding of the intrinsically disordered dehydrin Lti30, a cold-induced plant stress protein, Plant Cell 23 (2011) 2391–2404.

[67] P. Castiglioni, D. Warner, R.J. Bensen, D.C. Anstrom, J. Harrison, M. Stoecker, M. Abad, G. Kumar, S. Salvador, R. D'Ordine, S. Navarro, S. Back, M. Fernandes, J.

Targolli, S. Dasgupta, C. Bonin, M.H. Luethy, J.E. Heard, Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions, Plant Physiol. 147 (2008) 446–455.

[68] Y. Sato, S. Yokoya, Enhanced tolerance to drought stress in transgenic rice plants overexpressing a small heat-shock protein, sHSP17.7, Plant Cell Rep. 27 (2008) 329–334.

[69] S.D. Tyerman, C.M. Niemietz, H. Bramley, Plant aquaporins: multifunctional water and solute channels with expanding roles, Funct. Plant Biol. 25 (2002) 173–194.

[70] G.P. Bienert, F. Chaumont, Plant aquaporins: roles in water homeostasis, nutrition, and signaling processes, in: M. Geisler, K. Venema (Eds.), Transporters and Pumps in Plant Signaling, vol. 7, Springer, Berlin Heidelberg, 2011, pp. 3–36.

[71] Y. Li, G.-X. Wang, M. Xin, H.-M. Yang, X.-J. Wu, T. Li, The parameters of guard cell calcium oscillation encodes stomatal oscillation and closure in Vicia faba, Plant Sci. 166 (2004) 415–421.

[72] X. Wang, Y. Li, W. Ji, X. Bai, H. Cai, D. Zhu, X.-L. Sun, L.-J. Chen, Y.-M. Zhu, A novel Glycine soja tonoplast intrinsic protein gene responds to abiotic stress and depresses salt and dehydration tolerance in transgenic Arabidopsis thaliana, J. Plant Physiol. 168 (2011) 1241–1248.

[73] R. Aharon, Y. Shahak, S. Wininger, R. Bendov, Y. Kapulnik, G. Galili, Over-expression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress, Plant Cell 15 (2003) 439–447.

[74] M. Katsuhara, K. Koshio, M. Shibasaka, Y. Hayashi, T. Hayakawa, K. Kasamo, Over-expression of a barley aquaporin increased the shoot/root ratio and raised salt sensitivity in transgenic rice plants, Plant Cell Physiol. 44 (2003) 1378–1383.

[75] L. Guo, Z.Y. Wang, H. Lin, W.E. Cui, J. Chen, M. Liu, Z.L. Chen, L.J. Qu, H. Gu, Expression and functional analysis of the rice plasma-membrane intrinsic protein gene family, Cell Res. 16 (2006) 277–286.

[76] N. Sade, M. Gebretsadik, R. Seligmann, A. Schwartz, R. Wallach, M. Moshelion, The role of Tobacco aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress, Plant Physiol. 152 (2010) 245–254.

[77] N. Sade, B.J. Vinocur, A. Diber, A. Shatil, G. Ronen, H. Nissan, R. Wallach, H. Karchi, M. Moshelion, Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol. 181 (2009) 651–661.

[78] E.P. Glenn, J.J. Brown, E. Blumwald, Salt tolerance and crop potential of halophytes, Crit. Rev. Plant Sci. 18 (1999) 227–255.

[79] D.C. Plett, I.S. Moller, Na$^+$ transport in glycophytic plants: what we know and would like to know, Funct. Plant Biol. 33 (2010) 612–626.

[80] S. Laurie, K.A. Feeney, F.J.M. Maathuis, P.J. Heard, S.J. Brown, R.A. Leigh, A role for HKT1 in sodium uptake by wheat roots, Plant J. 32 (2002) 139–149.

[81] H. Shi, B.-h. Lee, S.-J. Wu, J.-K. Zhu, Overexpression of a plasma membrane Na$^+$/H$^+$ antiporter gene improves salt tolerance in Arabidopsis thaliana, Nat. Biotechnol. 21 (2003) 81–85.

[82] F. Zhao, S. Guo, H. Zhang, Y. Zhao, Expression of yeast SOD2 in transgenic rice results in increased salt tolerance, Plant Sci. 170 (2006) 216–224.

[83] M.P. Apse, G.S. Aharon, W.A. Snedden, E. Blumwald, Salt tolerance conferred by overexpression of a vacuolar Na$^+$/H$^+$ antiport in Arabidopsis, Science 285 (1999) 1256–1258.

[84] M.P. Apse, E. Blumwald, Engineering salt tolerance in plants, Curr. Opin. Plant Biol. 13 (2002) 146–150.

[85] H.-X. Zhang, J.N. Hodson, J.P. Williams, E. Blumwald, Engineering salt-tolerant Brassica plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12832–12836.

[86] C. He, J. Yan, G. Shen, L. Fu, A.S. Holaday, D. Auld, E. Blumwald, H. Zhang, Expression of an Arabidopsis vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field, Plant Cell Physiol. 46 (2005) 1848–1854.

[87] Z.-Y. Xue, D.-Y. Zhi, G.-P. Xue, H. Zhang, Y.-X. Zhao, G.-M. Xia, Enhanced salt tolerance of transgenic wheat (Tritivum aestivum L.) expressing a vacuolar Na$^+$/H$^+$ antiporter gene with improved grain yields in saline soils in the field and a reduced level of Na$^+$, Plant Sci. 167 (2004) 849–859.

[88] A.F. Yang, X.G. Duan, X.F. Gu, F. Gao, J.R. Zhang, Efficient transformation of beet (Beta vulgaris) and production of plants with improved salt-tolerance, Plant Cell Tissue Org. 83 (2005) 259–270.

[89] J. Zhao, D. Zhi, Z. Xue, H. Liu, G. Xia, Enhanced salt tolerance of transgenic progeny of tall fescue (Festuca arundinacea) expressing a vacuolar Na$^+$/H$^+$ antiporter gene from Arabidopsis, J. Plant Physiol. 164 (2007) 1377–1383.

[90] M.P. Apse, J.B. Sottosanto, E. Blumwald, Vacuolar cation/H$^+$ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of AtNHX1, the Arabidopsis vacuolar Na$^+$/H$^+$ antiporter, Plant J. 36 (2003) 229–239.

[91] M. Chen, Q.-J. Chen, X.-G. Niu, R. Zhang, H.-Q. Lin, C.-Y. Xu, X.-C. Wang, G.-Y. Wang, J. Chen, Expression of OsNHX1 gene in maize confers salt tolerance and promotes plant growth in the field, Plant, Soil Environ. 53 (2007) 490–498.

[92] H. Liu, Q. Wang, M. Yu, Y. Zhang, Y. Wu, H. Zhang, Transgenic salt-tolerant sugar beet (Beta vulgaris L.) constitutively expressing an Arabidopsis thaliana vacuolar Na$^+$/H$^+$ antiporter gene, AtNHX3, accumulates more soluble sugar but less salt in storage roots, Funct. Plant Biol. 31 (2008) 1325–1334.

[93] E. Bassil, M. Ohto, T. Esumi, H. Tajima, Z. Zhu, O. Cagnac, M. Belmonte, Z. Peleg, T. Yamaguchi, E. Blumwald, The Arabidopsis intracellular Na$^+$/H$^+$ antiporters NHX5 and NHX6 are endosome associated and necessary for plant growth and development, Plant Cell 23 (2011) 224–239.

[94] M. Li, X. Lin, H. Li, X. Pan, G. Wu, Overexpression of AtNHX5 improves tolerance to both salt and water stress in rice (Oryza sativa L.), Plant Cell Tissue Org. (2011), doi: 10.1007/s11240-011-9979-6.

[95] M. Li, Y. Li, H. Li, G. Wu, Overexpression of AtNHX5 improves tolerance to both salt and drought stress in Broussonetia papyrifera (L.) Vent, Tree Physiol. 31 (2011) 349–357.

[96] K. Yamaguchi-Shinozaki, K. Shinozaki, Transcriptional regulatory networks in cellular responses and toletance to dehydration and cold stresses, Annu. Rev. Plant Biol. 57 (2006) 781–803.

[97] R. Mantovani, The molecular biology of the CCAAT-binding factor NF-Y, Gene 239 (1999) 15–27.

[98] D.E. Nelson, P.P. Repetti, T.R. Adams, R.A. Creelman, J. Wu, D.C. Warner, D.C. Anstrom, R.J. Bensen, P.P. Castiglioni, M.G. Donnarummo, B.S. Hinchey, R.W. Kumimoto, D.R. Maszle, R.D. Canales, K.A. Krolikowski, S.B. Dotson, N. Gutterson, O.J. Ratcliffe, J.E. Heard, Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 16450–16455.

[99] H. Takasaki, K. Maruyama, S. Kidokoro, Y. Ito, Y. Fujita, K. Shinozaki, K. Yamaguchi-Shinozaki, K. Nakashima, The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice, Mol. Genet. Genomics 284 (2010) 173–183.

[100] H. Hu, M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang, L. Xiong, Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12987–12992.

[101] H. Hu, J. You, Y. Fang, X. Zhu, Z. Qi, L. Xiong, Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice, Plant Mol. Biol. 67 (2008) 169–181.

[102] J.S. Jeong, Y.S. Kim, K.H. Baek, H. Jung, S.-H. Ha, Y. Do Choi, M. Kim, C. Reuzeau, J.-K. Kim, Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions, Plant Physiol. 153 (2010) 185–197.

[103] K. Yamaguchi-Shinozaki, K. Shinozaki, A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress, Plant Cell 6 (1994) 251–264.

[104] K.R. Jaglo-Ottosen, S.J. Gilmour, D.G. Zarka, O. Schabenberger, M.F. Thomashow, Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance, Science 280 (1998) 104–106.

[105] Q. Liu, M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki, K. Shinozaki, Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis, Plant Cell 10 (1998) 1391–1406.

[106] M. Kasuga, Q. Liu, S. Miura, K. Yamaguchi-Shinozaki, K. Shinozaki, Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor, Nat. Biotechnol. 17 (1999) 287–291.

[107] S.J. Gilmour, A.M. Sebolt, M.P. Salazar, J.D. Everard, M.F. Thomashow, Over-expression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation, Plant Physiol. 124 (2000) 1854–1865.

[108] J.G. Dubouzet, Y. Sakuma, Y. Ito, M. Kasuga, E.G. Dubouzet, S. Miura, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression, Plant J. 33 (2003) 751–763.

[109] K.R. Jaglo, S. Kleff, K.L. Amundsen, X. Zhang, V. Haake, J.Z. Zhang, T. Deits, M.F. Thomashow, Components of the Arabidopsis C-Repeat/dehydration-responsive element binding factor cold-response pathway are conserved in Brassica napus and other plant species, Plant Physiol. 127 (2001) 910–917.

[110] Y. Ito, K. Katsura, K. Maruyama, T. Taji, M. Kobayashi, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice, Plant Cell Physiol. 47 (2006) 141–153.

[111] A. Karaba, S. Dixit, R. Greco, A. Aharoni, K.R. Trijatmiko, N. Marsch-Martinez, A. Krishnan, K.N. Nataraja, M. Udayakumar, A. Pereira, Improvement of water use efficiency in rice by expression of HARDY, an Arabidopsis drought and salt tolerance gene, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 15270–15275.

[112] J.L. Araus, G.A. Slafer, M.P. Reynolda, C. Royo, Plant breeding and drought in C$_3$ cereals: what should we breed for? Ann. Bot. 89 (2002) 925–940.

[113] Z. Peleg, E. Blumwald, Hormone balance and abiotic stress tolerance in crop plants, Curr. Opin. Plant Biol. 14 (2011) 290–295.

[114] T. Kiba, H. Sakakibara, Role of Cytokinin in the Regulation of Plant Development, in: E.C. Pua, M.R. Davey (Eds.), Plant Developmental Biology — Biotechnological Perspectives, vol. 2, Springer, Berlin Heidelberg, 2010, pp. 237–254.

[115] C.T. Argueso, T. Raines, J.J. Kieber, Cytokinin signaling and transcriptional networks, Curr. Opin. Plant Biol. 13 (2010) 533–539.

[116] T. Werner, T. Schmülling, Cytokinin action in plant development, Curr. Opin. Plant Biol. 12 (2009) 527–538.

[117] A. Santner, M. Estelle, Recent advances and emerging trends in plant hormone signalling, Nature 459 (2009) 1071–1078.

[118] A. Santner, L.I.A. Calderon-Villalobos, M. Estelle, Plant hormones are versatile chemical regulators of plant growth, Nat. Chem. Biol. 5 (2009) 301–307.

[119] S. Yamaguchi, Gibberellin metabolism and its regulation, Annu. Rev. Plant Biol. 59 (2008) 225–251.

[120] S.R. Cutler, P.L. Rodriguez, R.R. Finkelstein, S.R. Abrams, Abscisic acid: emergence of a core signaling network, Annu. Rev. Plant Biol. 61 (2010) 651–679.

[121] U.K. Divi, P. Krishna, Brassinosteroid: a biotechnological target for enhancing crop yield and stress tolerance, N. Biotechnol. 26 (2009) 131–136.

[122] J.P. Klingler, G. Batelli, J.-K. Zhu, ABA receptors: the start of a new paradigm in phytohormone signalling, J. Exp. Bot. 61 (2010) 3199–3210.

[123] S. Perilli, L. Moubayidin, S. Sabatini, The molecular basis of cytokinin function, Curr. Opin. Plant Biol. 13 (2010) 21–26.

[124] A.N. Stepanova, J.M. Alonso, Ethylene signaling and response: where different regulatory modules meet, Curr. Opin. Plant Biol. 12 (2009) 548–555.

[125] T.-W. Kim, Z.-Y. Wang, Brassinosteroid signal transduction from receptor kinases to transcription factors, Annu. Rev. Plant Biol. 61 (2010) 681–704.

[126] Y. Jaillais, J. Chory, Unraveling the paradoxes of plant hormone signaling integration, Nat. Struct. Mol. Biol. 17 (2010) 642–645.

[127] C. Wasternack, Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development, Ann. Bot. 100 (2007) 681–697.

[128] A. Santner, M. Estelle, The ubiquitin–proteasome system regulates plant hormone signaling, Plant J. 61 (2010) 1029–1040.

[129] J.I. Schroeder, J.M. Kwak, G.J. Allen, Guard cell abscisic acid signalling and engineering drought hardiness in plants, Nature 410 (2001) 327–330.

[130] S. Wilkinson, W.J. Davies, Drought, ozone, ABA and ethylene: new insights from cell to plant to community, Funct. Plant Biol. 33 (2010) 510–525.

[131] D. Huang, W. Wu, S.R. Abrams, A.J. Cutler, The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors, J. Exp. Bot. 59 (2008) 2991–3007.

[132] B.-Z. Xiao, X. Chen, C.-B. Xiang, N. Tang, Q.-F. Zhang, L.-Z. Xiong, Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions, Mol. Plant 2 (2009) 73–83.

[133] A.J. Thompson, J. Andrews, B.J. Mulholland, J.M.T. McKee, H.W. Hilton, J.S. Horridge, G.D. Farquhar, R.C. Smeeton, I.R.A. Smillie, C.R. Black, I.B. Taylor, Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion, Plant Physiol. 143 (2007) 1905–1917.

[134] Y. Wang, J. Ying, M. Kuzma, M. Chalifoux, A. Sample, C. McArthur, T. Uchacz, C. Sarvas, J. Wan, D.T. Dennis, P. McCourt, Y. Huang, Molecular tailoring of farnesylation for plant drought tolerance and yield protection, Plant J. 43 (2005) 413–424.

[135] L. Zhang, S. Xiao, W. Li, W. Feng, J. Li, Z. Wu, X. Gao, F. Liu, M. Shao, Overexpression of a Harpin-encoding gene *hrf1* in rice enhances drought tolerance, J. Exp. Bot. 62 (2011) 4229–4238.

[136] C.T. Argueso, F.J. Ferreira, J.J. Kieber, Environmental perception avenues: the interaction of cytokinin and environmental response pathways, Funct. Plant Biol. 32 (2009) 1147–1160.

[137] P.D. Hare, W.A. Cress, J. van Staden, The involvement of cytokinins in plant responses to environmental stress, Plant Growth Regul. 23 (1997) 79–103.

[138] S. Alvarez, E.L. Marsh, S.G. Schroeder, D.P. Schachtman, Metabolomic and proteomic changes in the xylem sap of maize under drought, Funct. Plant Biol. 31 (2008) 325–340.

[139] S. Gan, R.M. Amasino, Making sense of senescence. Molecular genetic regulation and manipulation of leaf senescence, Plant Physiol. 113 (1997) 313–319.

[140] T. Kunkel, Q.-W. Niu, Y.-S. Chan, N.-H. Chua, Inducible isopentenyl transferase as a high-efficiency marker for plant transformation, Nat. Biotechnol. 17 (1999) 916–919.

[141] Q.-H. Ma, Genetic engineering of cytokinins and their application to agriculture, Crit. Rev. Biotechnol. 28 (2008) 213–232.

[142] Z. Peleg, M. Reguera, E. Tumimbang, H. Walia, E. Blumwald, Cytokinin mediated source/sink modifications improve drought tolerance and increases grain yield in rice under water stress, Plant Biotechnol. J. 9 (2011) 747–758.

[143] R.M. Rivero, J. Gimeno, A. Van Deynze, H. Walia, E. Blumwald, Enhanced cytokinin synthesis in tobacco plants expressing $P_{SARK}::IPT$ prevents the degradation of photosynthetic protein complexes during drought, Plant Cell Physiol. 51 (2010) 1929–1941.

[144] R.M. Rivero, M. Kojima, A. Gepstein, H. Sakakibara, R. Mittler, S. Gepstein, E. Blumwald, Delayed leaf senescence induces extreme drought tolerance in a flowering plant, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19631–19636.

[145] R.M. Rivero, V. Shulaev, E. Blumwald, Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit, Plant Physiol. 150 (2009) 1530–1540.

[146] P. Zhang, W.-Q. Wang, G.-L. Zhang, M. Kaminek, P. Dobrev, J. Xu, W. Gruissem, Senescence-inducible expression of *isopentenyl transferase* extends leaf life, increases drought stress resistance and alters cytokinin metabolism in cassava, J. Integr. Plant Biol. 52 (2010) 653–669.

[147] M.E. Ghanem, A. Albacete, A.C. Smigocki, I. Frébort, H. Pospíšilová, C. Martínez-Andújar, M. Acosta, J. Sánchez-Bravo, S. Lutts, I.C. Dodd, F. Pérez-Alfocea, Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato, J. Exp. Bot. 62 (2011) 125–140.

[148] H. Stein, A. Honig, G. Miller, O. Erster, H. Eilenberg, L.N. Csonka, L. Szabados, C. Koncz, A. Zilberstein, Elevation of free proline and proline-rich protein levels by simultaneous manipulations of proline biosynthesis and degradation in plants, Plant Sci. 181 (2011) 140–150.

[149] B. Le Martret, M. Poage, K. Shiel, G.D. Nugent, P.J. Dix, Tobacco chloroplast transformants expressing genes encoding dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase, exhibit altered anti-oxidant metabolism and improved abiotic stress tolerance, Plant Biotechnol. J. 9 (2011) 661–673.

[150] Y.P. Lee, K.-H. Baek, H.-S. Lee, S.-S. Kwak, J.-W. Bang, S.-Y. Kwon, Tobacco seeds simultaneously over-expressing Cu/Zn-superoxide dismutase and ascorbate peroxidase display enhanced seed longevity and germination rates under stress conditions, J. Exp. Bot. 61 (2010) 2499–2506.

[151] I.R. Henderson, S.E. Jacobsen, Epigenetic inheritance in plants, Nature 447 (2007) 418–424.

[152] M.-T. Hauser, W. Aufsatz, C. Jonak, C. Luschnig, Transgenerational epigenetic inheritance in plants, BBA-Gene Regul. Mech. 1809 (2011) 459–468.

[153] S. Feng, S.E. Jacobsen, W. Reik, Epigenetic reprogramming in plant and animal development, Science 330 (2010) 622–627.

[154] O. Dyachenko, N. Zakharchenko, T. Shevchuk, H. Bohnert, J. Cushman, Y. Buryanov, Effect of hypermethylation of CCWGG sequences in DNA of *Mesembryanthemum crystallinum* plants on their adaptation to salt stress, Biochemistry (Mosc.) 71 (2006) 461–465.

[155] L. Zhong, Y. Xu, J. Wang, The effect of 5-azacytidine on wheat seedlings responses to NaCl stress, Biol. Plant. 54 (2010) 753–756.

[156] G.S. Scippa, M. Di Michele, E. Onelli, G. Patrignani, D. Chiatante, E.A. Bray, The histone-like protein H1-S and the response of tomato leaves to water deficit, J. Exp. Bot. 55 (2004) 99–109.

[157] D. Baulcombe, RNA silencing in plants, Nature 431 (2004) 356–363.

[158] B. Khraiwesh, J.-K. Zhu, J. Zhu, Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants, BBA-Gene Regul. Mech. (2011), doi:10.1016/j.bbagrm. 2011.05.001.

[159] R. Sunkar, A. Kapoor, J.-K. Zhu, Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by downregulation of miR398 and important for oxidative stress tolerance, Plant Cell 18 (2006) 2051–2065.

[160] X. Zhang, Z. Zou, P. Gong, J. Zhang, K. Ziaf, H. Li, F. Xiao, Z. Ye, Over-expression of microRNA169 confers enhanced drought tolerance to tomato, Biotechnol. Lett. 33 (2011) 403–409.

[161] P. Gao, X. Bai, L. Yang, D. Lv, Y. Li, H. Cai, W. Ji, D. Guo, Y. Zhu, Over-expression of *osa-MIR396c* decreases salt and alkali stress tolerance, Planta 231 (2010) 991–1001.

[162] R. Sunkar, MicroRNAs with macro-effects on plant stress responses, Semin. Cell Dev. Biol. 21 (2010) 805–811.

[163] R. Schwab, J.F. Palatnik, M. Riester, C. Schommer, M. Schmid, D. Weigel, Specific effects of microRNAs on the plant transcriptome, Dev. Cell 8 (2005) 517–527.

[164] E.A. Ainsworth, D.R. Bush, Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity, Plant Physiol. 155 (2011) 64–69.

[165] J.R. Evans, Photosynthesis and nitrogen relationships in leaves of C_3 plants, Oecologia 78 (1989) 9–19.

[166] K. Hikosaka, I. Terashima, Nitrogen partitioning among photosynthetic components and its consequence in sun and shade plants, Func. Ecol. 10 (1996) 335–343.

[167] C.H. Foyer, S. Shigeoka, Understanding oxidative stress and antioxidant functions to enhance photosynthesis, Plant Physiol. 155 (2011) 93–100.

[168] R.F. Sage, The evolution of C_4 photosynthesis, New Phytol. 161 (2004) 341–370.

[169] J.M. Hibberd, J.E. Sheehy, J.A. Langdale, Using C_4 photosynthesis to increase the yield of rice—rationale and feasibility, Curr. Opin. Plant Biol. 11 (2008) 228–231.

[170] R.F. Sage, X.-G. Zhu, Exploiting the engine of C_4 photosynthesis, J. Exp. Bot. 62 (2011) 2989–3000.

[171] M.S.B. Ku, D. Cho, U. Ranade, T.P. Hsu, X. Li, D.M. Jiao, J. Ehleringer, M. Miyao, M. Matsuoka, Photosynthetic performance of transgenic rice plants overexpressing maize C_4 photosynthesis enzymes, in: J.E. Sheehy, P.L. Mitchell, B. Hardy (Eds.), Studies in Plant Science, 7, 2000, pp. 193–204.

[172] M. Matsuoka, R.T. Furbank, H. Fukayama, M. Miyao, Molecular engineering of C_4 photosynthesis, Annu. Rev. Plant Physiol. 52 (2001) 297–314.

[173] D. Jiao, X. Huang, X. Li, W. Chi, T. Kuang, Q. Zhang, M. Ku, D. Cho, Photosynthetic characteristics and tolerance to photo-oxidation of transgenic rice expressing C_4 photosynthesis enzymes, Photosynth. Res. 72 (2002) 85–93.

[174] M.S.B. Ku, D. Cho, X. Li, D.M. Jiao, M. Pinto, M. Miyao, M. Matsuoka, Introduction of Genes Encoding C_4 Photosynthesis Enzymes into Rice Plants: Physiological Consequences, in: J.A. Goode, D. Chadwick (Eds.), Novartis Foundation Symposium 236 — Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality, John Wiley & Sons, Ltd., Chichester, UK, 2007, pp. 100–116.

[175] R.E. Häusler, H.J. Hirsch, F. Kreuzaler, C. Peterhänsel, Overexpression of C_4-cycle enzymes in transgenic C_3 plants: a biotechnological approach to improve C_3-photosynthesis, J. Exp. Bot. 53 (2002) 591–607.

[176] T. Rademacher, R.E. Häusler, H.-J. Hirsch, L. Zhang, V. Lipka, D. Weier, F. Kreuzaler, C. Peterhänsel, An engineered phosphoenolpyruvate carboxylase redirects carbon and nitrogen flow in transgenic potato plants, Plant J. 32 (2002) 25–39.

[177] C.-J. Ruan, H.-B. Shao, J.A. Teixeira da Silva, A critical review on the improvement of photosynthetic carbon assimilation in C_3 plants using genetic engineering, Crit. Rev. Biotechnol. (2011), doi:10.3109/07388551.07382010.07533119.

[178] R.T. Furbank, S. von Caemmerer, J. Sheehy, G. Edwards, C_4 rice: a challenge for plant phenomics, Funct. Plant Biol. 36 (2009) 845–856.

[179] T.L. Sage, R.F. Sage, The functional anatomy of rice leaves: implications for refixation of photorespiratory CO_2 and efforts to engineer C_4 photosynthesis into rice, Plant Cell Physiol. 50 (2009) 756–772.

[180] C. Peterhansel, Best practice procedures for the establishment of a C_4 cycle in transgenic C_3 plants, J. Exp. Bot. 62 (2011) 3011–3019.

[181] E.H. Murchie, M. Pinto, P. Horton, Agriculture and the new challenges for photosynthesis research, New Phytol. 181 (2009) 532–552.

[182] J.T. Odell, F. Nagy, N.-H. Chua, Identification of DNA sequences required for activity of the cauliflower mosaic virus *35S* promoter, Nature 313 (1985) 810–812.

[183] S. Holtorf, K. Apel, H. Bohlmann, Comparison of different constitutive and inducible promoters for the overexpression of transgenes in *Arabidopsis thaliana*, Plant Mol. Biol. 29 (1995) 637–646.

[184] D. McElroy, M. Rothenberg, R. Wu, Structural characterization of a rice actin gene, Plant Mol. Biol. 14 (1990) 163–171.

[185] T.-H. Hsieh, J.-t. Lee, Y.-y. Charng, M.-T. Chan, Tomato plants ectopically expressing *Arabidopsis CBF1* show enhanced resistance to water deficit stress, Plant Physiol. 130 (2002) 618–626.

[186] N. Ori, M.T. Juarez, D. Jackson, J. Yamaguchi, G.M. Banowetz, S. Hake, Leaf senescence is delayed in Tobacco plants expressing the maize homeobox gene *knotted1* under the control of a senescence-activated promoter, Plant Cell 11 (1999) 1073–1080.

[187] M. Wassenegger, G. Krczal, Nomenclature and functions of RNA-directed RNA polymerases, Trends Plant Sci. 11 (2006) 142–151.

[188] A. Dietz-Pfeilstetter, Stability of transgene expression as a challenge for genetic engineering, Plant Sci. 179 (2010) 164–167.

[189] I.S. Moller, M. Gilliham, D. Jha, G.M. Mayo, S.J. Roy, J.C. Coates, J. Haseloff, M. Tester, Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na^+ transport in *Arabidopsis*, Plant Cell 21 (2009) 2163–2178.

[190] S.L. Zeller, O. Kalinina, S. Brunner, B. Keller, B. Schmid, Transgene × environment interactions in genetically modified wheat, PLoS ONE 5 (2010) e11405.