Overview on Crop Genetic Engineering for Drought-prone Environments

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Population growth and climate change present crop researchers and plant breeders with one of the great grand challenges of the 21st century - to productively grow nutritious crops in water-scarce environments (Pimentel et al. 2004). Agriculture currently uses 75% of the total global consumption of water (Molden 2007). Some of the crop technologies that were able to feed the growing world in the 20th century were highly dependent on inputs including water resources, whose use in agriculture almost treble from the beginning of the 1940s to the end of the century. About a third of the current global population lives in water-stressed locations and this may increase to two thirds within the next 25 years. Consumptive water use (or transpired water) by all food and fodder crops will, therefore, need to increase from its present estimated level (7,000-12,586 km^3 year) to be capable of feeding adequately the 9.3 billion population of 2050 (Falkenmark and Rockström 2004). Water use efficiency varies substantially between crops, for example, to produce 1 kg of grain on average requires 900 liters for wheat (*Triticum* spp.), 1400 liters for maize (*Zea mays*) and 1900 liters for rice (*Oriza sativa*) (Pimentel 1997). In addition, there are great prospects for increasing the water use efficiency of specific genotypes within each crop.

Water use-efficiency and water productivity are being sought by agricultural researchers worldwide to address the global challenge that especially afflicts the resource poor, in drought-prone environments across the developing world. Under water-scarcity, grain yields of cereals such as wheat are a function of the amount of water used by the crop, how efficiently the crop uses this water for biomass-growth (i.e., water-use efficiency or above-ground biomass/water use), and the harvest index; i.e., the proportion of grain yield to above ground biomass (Passioura 1977). Water use efficiency (WUE) is the ratio of total dry matter accumulation to evapo-transpiration and other water losses; i.e., water entering and lost from the system that is not transpired through the plant. An increase in transpiration efficiency or a reduction in soil evaporation will increase WUE. More recently, water productivity (WP) has been defined at the crop level as the ratio of biomass with economic value (for example grain yield of cereals) compared to the amount of water transpired (WPT) (Bouman 2007). This WP has been labeled as

''productive'' because transpiration is the only water flow in a field actually passing through the crop. Both WUE and WP may be improved through plant breeding, as can biomass accumulation and harvest index (Parry et al. 2005).

Sub-Saharan Africa, in particular, possesses the smallest ratio of irrigated to rainfed agriculture, followed by Latin America, the Middle East and North Africa, whereas Asia has the highest proportion of irrigated land. In rainfed areas, water availability is limited and unpredictable, and indications are that climate change is making this variability more extreme. Climate change will further exacerbate the water crisis by causing a decline in water run-off in many regions. This will be especially severe in developing world environments where rainfall is highly variable and soils are degraded. North, Eastern and Southern Africa as well as West, South and Far East Asia will be among the most water-vulnerable regions of the world in 2025 (Rijsberman 2006 and references therein). In all these regions maize and wheat are among the main staple crops, which are grown mostly in rainfed environments by smallholder farmers. The demand for both cereal crops will also increase over the next 20 years with global demand for maize as feed increasing more rapidly than its food use whereas most of the world's wheat grain harvests will continue to be used for human consumption (CIMMYT 2005). Hence, agricultural researchers are seeking new genetic enhancement and natural resource management options that will help to ensure maize and wheat productivity can continue supplying sufficient food to feed the increasing human population.

The status of maize and wheat breeding under water scarcity

Conventional and genomics-assisted breeding of maize for drought-prone environments. Drought is an intractable problem that puts the livelihoods of millions of poor at risk every crop season. Conventional breeding and marker-assisted selection offer an opportunity for significant and predictable incremental improvements in the drought tolerance of new maize cultivars (Banziger and Araus 2007). In this regard, significant progress in grain yield under drought stress has been made through selection in multi-environment trials for component traits such as kernel set, rapid silk exertion, and reduced barrenness (Campos et al. 2004). Likewise, the genetic dissection of maize performance in drought-prone environments has greatly benefited from the use of DNA markers (Ribaut et al. 1996, 1997; Ribaut and Ragot 2007; Tuberosa et al. 2002). More than a decade of this research using 10 segregating populations, as well as $F_{2/3}$, $F_{3/4}$ and recombinant inbred lines tested at 30 stress-prone environments in Mexico, Kenya and Zimbabwe, to evaluate about 350 morphological characters and 70 physiological traits, led to the

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identification of about 3000 quantitative trait loci (QTL) data points. This research showed that backcross breeding can be used for introgressing a few target QTL into an elite maize line. However, backcrossing does not appear to be a very effective breeding approach in maize when many QTL of small effect are involved. Furthermore, QTL are often germplasm-specific and the costs for marker-aided selection (MAS) for many QTL of small effect may be higher than those from conventional cross-breeding of maize. Thus, the challenge for molecular breeders is to identify QTL of major effect that are independent of genetic background and to devise more effective breeding approaches for the application of the resultant markers such a pedigree selection. This approach has shown promise in rice (Bernier et al. 2007).

CIMMYT initiated in 1997 a client-oriented breeding undertaking targeted at improving maize for the drought-prone mid-altitudes of sub-Saharan Africa (Bänziger et al. 2006). As a result of this innovative approach more than 50 new cultivars were released, which are now grown on at least 1 million ha in Southern Africa (Fig 1.). This work produced new genotypes not only for drought-prone environments but also tolerant to low soil fertility, parasitic weeds, and other common plant stresses. It also revolutionized the way in which new maize cultivars are bred in Africa. A series of so-called "mother-baby" trials jointly managed by researchers and farmers were used to evaluate the breeding materials under farmers' conditions. The "mother" trial may involve as many as 12 breeding materials sown under varied researcher-designed treatments. The mother trial is located close to the community and is managed by schools, colleges, or extension agencies. The "babies" are satellite subsets of the mother trial, comprising approximately four to six cultivars in the fields of participating farmers using their own inputs and equipment. This approach was a fundamental change from the customary practice of testing new breeding materials under well-fertilized and well-irrigated conditions. In the "mother and baby" trials system CIMMYT researchers, national agricultural research centers, and private sector partners created a network of regional "stress breeding" sites that, for the first time, provide objective information on how well maize cultivars perform under conditions faced by poor farmers, especially drought- prone infertile soils. By working in partnership with local communities and by replicating the poor conditions found in farmers' fields, the approach was tailored to meet the needs of poor farmers who had not previously benefited from conventional breeding programs. In 2006, the King Baudouin Award, the highest accolade of the Consultative Group on International Agricultural Research (CGIAR), was bestowed to CIMMYT and partners for this maize breeding success story.

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Wheat improvement for water-limiting environments. Globally, almost 50% of the wheat cultivated in the developing world (50 million ha) is sown under rainfed systems that receive less than 600 mm per annum, the poorest and most disadvantaged farmers of whom live in areas of less than 350 mm per annum (CIMMYT 2005). Furthermore, even in traditionally irrigated areas, supplementary rather than full irrigation is becoming common, exposing wheat systems to water stress at various stages during the season. Water productivity is therefore an increasingly important trait for wheat cultivars for irrigated areas.

Recognizing WP and WUE as priorities for wheat, CIMMYT has worked to disaggregate grain yield under water stress into distinct components and to apply those findings to the genetic enhancement of this crop (Reynolds and Borlaug 2006). Ongoing research is providing a better understanding of traits with major effects on water productivity in dryland wheat areas. These include root architecture and physiological traits, resistance to soil-borne pests and diseases, tolerance to heat and salinity, zinc deficient and boron toxic soils. The combination of improved germplasm, the Center and partners' expertise in drought physiology, soil-borne diseases, and agronomy, and the availability of markers for various traits place CIMMYT in a unique position to develop water-productive wheat with resistance to the important stresses for use by partners throughout the developing world.

Some important attributes for drought-prone environments are available in the wild relatives of wheat (Reynolds et al. 2007). Re-synthesizing hexaploid wheat with wild ancestors has been used at CIMMYT for tapping this useful variation and incorporating such genetic resources into wheat-bred germplasm (Fig. 2). In addition, transgenic approaches for incorporating stress inducible regulatory genes that encode proteins such as transcription factors (e.g. *DREB1A*) into the wheat cultigen pool are also being pursued and details are provided below.

Gene regulation during plant response to environmental stress

Conventional breeding and marker-assisted selection have been important mechanisms for achieving yield improvements under drought-prone environments for most crops (Bennett 2003). However, transgenic technologies promise a much more targeted and design-led approach, although not without certain biological uncertainties and potential risks during product development and deployment. In this regard, generating plants with catch-all

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alterations involving the signaling pathways and their early responses that are common to several abiotic stresses such as heat, cold, salinity or water scarcity caught research attention worldwide (Tester and Bacic 2005). For example, microarray analysis using rice cDNAs was able to identify stress-inducible genes in response to dehydration, high salt, and low temperature in rice (Rabbani et al. 2003). The stress-inducible expression of the candidate genes identified by this analysis was confirmed using RNA gel-blot analysis. A total of 73 genes appear to be stress-inducible genes in rice (Yamaguchi-Shinozaki and Maruyama 2004), However, it is likely that it will be necessary to reconstruct the gene and metabolic networks and to verify the roles of specific transcription factors through transformation studies before we have sufficient information about regulatory genes to enable us to routinely manipulate them effectively in plant stress biology at the system level (Chen and Zhu 2004).

Molecular biology research has showed that several genes with distinct functions are induced by environmental stresses such as drought, salinity and low temperature in plants (Agarwal et al. 2006, Myzoguchi et al. 1996, Nakashima and Yamaguchi-Shinozaki 2005). Thus, studies have been carried out in the model system *Arabidopsis thaliana*, to alter expression levels or patterns for transcription factors involved in the early responses to stresses (Seki et al. 2003, Xiong et al. 2002). For example, the C-repeat dehydration responsive element (DRE) is a cis-acting DNA regulatory element in plants that stimulates transcription in response to stresses such as low temperatures or water deficit in *A. thaliana* (Yamaguchi-Shinozaki and Shinozaki 1994, Stockinger et al. 1997).

Abscisic acid (ABA), a phytohormone, plays important roles in seed maturation and dormancy as well as in the adaptation of vegetative tissues to abiotic environmental stresses such as drought and high salinity. At least four independent signaling pathways function in the induction of stress-inducible genes in response to dehydration in *Arabidopsis* (Gilmour et al. 1998). Two are ABA-dependent and two are ABA-independent. ABA-inducible genes may contain a conserved element named ABA-responsive element (ABRE) (Py-ACGTGG/TC) in their promoter regions, which functions as a cis-acting element and is involved in ABA-responsive gene expression (Himmelbach et al. 2003). Recently, Furihata et al. (2006) found that the ABA-dependent multi-site phosphorylation of *AREB1* regulates its own activation in plants.

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The induction of the dehydration-responsive *Arabidopsis* gene, *rd29B*, is mediated mainly by ABA (Uno et al. 2000), whereas the stress-induced gene *rd29A* is induced through the ABA-independent pathway (Liu et al. 1998). The *Dehydration-Responsive Element Binding* gene *1* (*DREB1*) and *DREB2* are transcription factors that bind to the promoter of genes such as *rd29A*, thereby inducing expression in response to drought, salt and cold (Dubouzet et al. 2003). Because *DREB1* binds to a DRE/CRT (dehydration-responsive element / C-repeat) *cis*-acting element, it was also termed as *DREB1/CBF* (DRE-binding protein 1/ C-repeat binding factor). It was recently reported that over-expression of *DREB2A CA* gene (an active form of *DREB2A* gene) induces not only drought- and salt-responsive genes but also heat-shock (HS)-related genes (Sakuma et al. 2006). The *DREB2A* up-regulated genes are classified into three groups based on their expression patterns: genes induced by HS, genes induced by drought stress, and genes induced by both HS and drought stress.

Searching for DREB-like genes in crop gene pools

Stress inducible regulatory genes that encode proteins which act as transcription factors (e.g. *DREB* genes from *Arabidopsis*) have been validated through over-expressed in transgenic plants, which show stress-tolerant phenotypes (Yamaguchi-Shinozaki and Shinozaki 2006). Thus, since regulatory genes appear to be an important component of plants response to environmental stresses, there have been many efforts to isolate abiotic stress-inducible transcription factors in other species, especially in important cereal crops.

In rice, one homolog, named *OsDREB2A*, was identified as a DREB2-type protein. Similar to *Arabidopsis DREB2A*, *OsDREB2A* was gradually induced by dehydration and high salinity stress, but hardly increased under cold stress (Dubouzet et al. 2003). A transcription factor, *OsDREB1B,* has been also isolated in rice, and shown to be over-expressed in transgenic *Arabidopsis* plants under cold and high-temperature and providing tolerance to those stresses (Qin et al. 2007b). More recently, gene expression of *OsNAC6* –another gene member of the NAC transcription factor gene family in rice, was induced by abiotic stresses such as cold, drought and high salinity, as well as by wounding and blast disease (Nakashima et al. 2007). Transgenic rice plants over-expressing *OsNAC6* constitutively showed an improved tolerance to dehydration and high-salt stresses, and also exhibited increased tolerance to blast disease but exhibited growth retardation and low reproductive yields.

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In wheat, the *TaDREB1* was found to be induced by cold, salinity and drought, and was classified as a DREB2-type transcription factor based on phylogenetic analysis (Shen et al. 2003). Similarly, a gene named *TdDRF1* has been isolated and characterized in durum wheat (*T. durum*) (Latini et al. 2007). This gene belongs to the *DREB* gene family and produces three forms of transcripts through alternative splicing: the truncated form *TdDRF1.2* was at all times the most expressed and together with that of *TdDRF1.3* transcript increased sharply after 4 days of dehydration, but then decreased at 7 days, whereas the *TdDRF1.1* transcript was the least expressed overall and less variable with the duration of dehydration. In barley, a DREB2-type protein *HvDRF1,* was also reported to accumulate under drought and salt stresses and was involved in ABA-mediated gene regulation (Xue and Loveridge 2004).

Qin et al. (2007a) cloned *ZmDREB2A,* a *DREB2* homolog from maize, whose transcripts were stimulated by cold, dehydration, salt and heat stresses in maize seedlings. Quantitative real-time PCR analyses demonstrated that functional transcription form of *ZmDREB2A* was significantly induced by stresses. Moreover, their research showed that constitutive or stress-inducible expression of *ZmDREB2A* resulted in improved drought stress tolerance. Finally, the authors indicated that over-expression of *ZmDREB2A* also enhanced thermo-tolerance in transgenic plants, which suggests it may play a dual functional role in mediating the expression of genes responsive to both water and heat stress. A cDNA with an open reading frame of 332 amino acids, encoding the transcription activation factor *DREB2A*, was cloned recently from pearl millet (*Pennisetum glaucum*), which is well known as one of the most stress tolerant food grain crop of the semi-arid tropics (Agarwal et al. 2007).

Three *DREB* homologue genes (*GmDREBa*, *GmDREBb* and *GmDREBc*) have also been isolated from soybean (*Glycine max*), and all of them bound to the dehydratation-responsive element (Li et al. 2005). More recently, the function of these genes has been validated through their over-expression in transgenic soybean plants where they conferred drought and salinity tolerance (Chen et al. 2007).

The above results show a consistent presence and role of *DREB*-like genes in abiotic stress responses across species. This suggests that transcription factors may be ubiquitous in higher plants, and reinforces the value of using plant model systems for identifying useful genes that can be implemented in the germplasm enhancement of food staples.

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Encouraged by these findings, researchers are also searching for other families of transcription factors in crop species to enhance their performance in stressful environments, e.g. NAM, ATAF and CUC (NAC) (Hu et al. 2006), or an intronless zinger-finger protein gene *(*Mukhopadhyay et al. 2004) in rice for saline-, drought-, and cold- prone cropping systems. The second gene is also induced by sub-emergence, heavy metals and injury. The use of such plant genes for transgenic crop improvement may also lead to a better social acceptance *viz.* a *viz.* the transgenic approach of inserting foreign gene constructs. Hence, geneticists should search for more *DREB*-like homolog(s) through allele mining in crop species of the semi-arid tropics such as millets or sorghum (*Sorghum bicolor*). However, it is critically important that this type of work is carried out in close collaboration with physiologists to ensure accurate and precise testing of their performance under appropriate water deficit profiles and other stresses. When such work is carried out under a holistic approach it is likely to open new paths for the genetic betterment of crop species for improved food security and enhanced livelihoods of poor farmers across the developing world.

Recent advances and future prospects for engineering cereal crops with *DREB-like genes*

Genetically engineered cultivars containing various gene constructs to enhance their performance under water stress will be an important future strategy for facilitating the production of cereals and other crops in drought-prone environments (Hoisington and Ortiz 2006). They will provide an attractive and complementary option for improving a plant's performance under stress conditions. Particularly attractive is the single, dominant nature of the transgene that makes the transfer and maintenance of this system in any cultivar much easier than conventional sources based on polygenes.

Crop engineering by inserting transcription factors will require both promoter and coding regions to ensure the functioning of those elements that govern cross-talk between different abiotic stresses and plant developmental "programs" (Bonhert et al. 2006). Transgenic rice with *AtDREB1A* or its rice orthologue *OsDREB1A* tested in pots showed improved tolerance to simulated drought, high-salt and low-temperature stresses (Yamaguchi-Shinozaki and Shinozaki 2004). Enhanced content of proline and soluble sugars was detected in this transgenic rice, together with altered transcript levels for various genes controlled by *DREB1A*. Likewise, the *DREB1.CBF* transcription factor was involved in cold-responsive expression in transgenic rice (Ito et al. 2006). Although these transgenic rice plants had improved tolerance to drought, high-salt and low-temperature stresses like the transgenic *Arabidopsis* plants

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over-expressing *OsDREB1* or *DREB1* they showed growth retardation under normal growth conditions. Resolving this type of issue will be fundamentally important for developing a viable transgenic drought tolerant new cultivar. Potential solutions include coupling *DREB*-like genes with a strong stress-inducible promoter to reduce the growth inhibition under unstressed conditions (Kasuga et al. 1999), *DREB1/CBF* genes have been successfully used to improve abiotic stress tolerance in a number of different plants (Nakashima and Yamaguchi-Shinozaki 2005). For example, enhanced tolerance to drought, cold and salt was observed in *Arabidopsis* plants in which the *AtDREB1A* gene was over-expressed under the control of the viral CaMV35S promoter. Alternatively, conventional breeding approaches may be able to identify background genotypes with good combining ability or modifier genes to counteract the yield penalty under optimum cropping conditions.

The Japan International Research Center for Agricultural Sciences (JIRCAS) has shared with some centers of the CGIAR gene constructs containing the *AtDREB1A* gene under the control of various promoters (Nakashima and Yamaguchi-Shinozaki 2005). These transgenes were introduced into several crops with the expectation that *AtDREB1A* would recognize the DRE of endogenous genes and enhance stress responsiveness. For example, different transgenic groundnut (*Arachis hypogaea*) lines were produced by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the transgenic lines showed delayed wilting under simulated drought by 20 to 25 days compared with non-transgenic controls (Bhatnagar-Mathur et al. 2004, 2007). Similarly, transgenic potato (*Solanum tuberosum*) bearing the *DREB1A* gene driven by the stress-inducible *rd29A promoter* increased salt-stress and freezing tolerance concomitant to its copy number (Behnam et al. 2006, 2007), which shows the versatile use of *DREB* transgenic technology to improve crops for growing in stressful environments.

*DREB*1A *in wheat.* Transgenic plants produced at CIMMYT were tested in small pots in biosafety greenhouses (Pellegrineschi et al. 2004). The *DREB1A* gene was placed under the control of a stress-inducible promoter from the *rd29A* gene and inserted via biolistic transformation into bread wheat (*T. aestivum*). The expression of the *actin* and *DREB1A* genes before and after water stress was determined in individual plants of T2 families by RT-PCR. The *actin* gene was constitutively expressed in all plants before and after water stress. Transcripts of the *DREB1A* gene driven by the *rd29A* promoter were detected after only 2 days of water stress. No differences in plant growth or in plant morphology related to the presence of the *DREB1A* were observed between the transgenic lines and controls

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during the first days of water stress. At the 4-5 leaf stage, the plants were subjected to increasing water stress by prolonged withholding of water. The control plants and transgenic plants were randomly distributed in the experiment. After 10 days without water, the control plants began to show water stress symptoms (loss of turgor and bleaching of the leaves). Plants expressing the *DREB1A* gene demonstrated substantial resistance to water stress in comparison with checks under experimental greenhouse conditions manifested by a 10-day delay in wilting when water was withheld (Fig. 3). Severe symptoms (death of all leaf tissue) were evident in the controls after 15 days without water. The transgenic wheat lines started to show water stress symptoms only after 15 days. In summary, tolerance to water stress was always associated with the presence of the *DREB1A* transgene.

The greenhouse pot experiments based on severe desiccation stress do not however represent typical field conditions and, therefore, the plants may not be exhibiting a response that would be valuable in farmers' fields. It has subsequently been demonstrated that this approach does not provide an effective pre-screening step for economic drought tolerance and should not be used for performance-based selection (Pellegrineschi, Reynolds and Wu, unpub. data). Unfortunately, molecular biology researchers are typically screening transgenic plants in small pots with unrealistically low volumes of soil, and often subjecting plants to rapid severe stress cycles –each ranging from an hour to just a few days. When severe stress is imposed rapidly, a greater number of plant responses will be associated with injury-induced and survival-related mechanisms than if the water deficit was slowly increased over a long-term. Hence, CIMMYT researchers shifted their attention to evaluating transgenic wheat lines in contained field trials. These screenhouse trials were the first time that transgenic wheat had been tested under field-like conditions in Mexico, and rigorous biosafety procedures were followed (Fig. 4). Crop canopy micro-climates in these studies were probably not highly representative of farmers' fields. However, the edaphic environment was much more realistic and the scale of the trial facilitated small plot studies rather than analyzing a few isolated plants in small pots.

Preliminary results showed that the *DREB1A* gene in wheat significantly lowers canopy temperature compared with the control in these trials mimicking unpredictable mid-season (vegetative phase) drought (Fig. 5). However, these effects were largely driven by differences in phenology. After extensive research on dehydration tolerance, it was clear that the gene *DREB1A* in wheat delayed development in the transgenic plants but did not result in larger

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biomass accumulation or better grain yields than the control under water stress. These results indicate that it is essential to move as quickly as possible to investigate the impact on both plant growth and grain yield of transgenic plants in replicated field trials under various appropriate drought stress profiles as well as to investigate at what stages of growth and in which genetic backgrounds the *DREB* gene may have its most significant effect.

Although the use of transgenics to provide enhanced grain yield under drought stress is still experimental in nature, CIMMYT researchers are optimizing both the transformation and screening methods to maximize potential success in a subsequent product development phase. It is now important for researchers to study gene epistasis (with different genetic backgrounds and with difference sources of drought tolerance) and genotype-by-environment interaction. In this regard, CIMMYT plans to test the other and hopefully more powerful *DREB* gene constructs (e.g. with drought-specific rather than stress-induced promoters) provided by JIRCAS (Fig. 6), In addition, CIMMYT researchers will study the effects of these different constructs in a variety of wheat genetic backgrounds, including germplasm developed through conventional breeding for drought-prone environment, to assess if the resulting plants can use water even more efficiently. Likewise, it will be interesting to assess if this *DREB* transgene would interact with known QTL crop genes that are revealed by genomics research, e.g. a QTL on the proximal region of the long arm of chromosome 4A, contributed by wheat cultivar Dharwar Dry, was found to have a significant impact on performance under reduced moisture (Kirigwi 2007). The *DREB* transgene may be inserted into wheat lines harboring this QTL and through appropriate experimental design and biometrics tools assess if such interaction will affect the action of *DREB*-transgenic wheat line(s) under water stress.

CIMMYT's wheat transgenic research will also give increasing emphasis to evaluation of physiological processes and traits that are known to influence abiotic stress tolerance in grasses. This will guide both conventional and molecular breeding for further genetic manipulations, particularly if the undertaking is framed by a systemic and iterative interface between plant physiology and genetics. For example, in many cropping systems drought stress tends to develop slowly as the soil dries. Plants that are subjected to drought conditions in this gradual manner accumulate solutes that maintain cell hydration, and undergo complex adjustments in their morphology and physiological characteristics. However, most experiments that have been published thus far are based on rapid, severe water-deficit treatments that are likely to completely miss this type of mechanism. It is, therefore, important

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that experiments be conducted under conditions that more closely approximate stress development in the field. Such an experiment will permit a better understanding of the potential functions of the introduced gene in stress tolerance. In particular, timing, duration and intensity of stress are the key elements for screening breeding materials in drought-mimicking environments. If the results from field trials properly taking into account such environmental factors are positive, then transgenic *DREB*-wheat will provide a powerful option for improving grain yield of wheat under water-stress conditions. Currently thinking suggests that *DREB* will be most useful for wheat breeding targeting cropping systems with unpredictable severe mid-season drought stress.

Biosafety and other risk assessments

Although there have been significant advances in transgenic research aimed at addressing abiotic stresses affecting crops worldwide, there are still some significant issues to address regarding product deployment. Genetically-modified crops with enhanced environmental stress tolerance require substantial advances in biosafety assessment and regulatory approval that are very different to the first generation of commercial transgenic crops with traits such as delayed ripening, herbicide tolerance, virus and insect resistance, or male-sterility (De Greef 2004). Genetically engineered crops for abiotic stress-prone environments pose new questions regarding safety and impact. For example, new phenotypes resulting from transgenic technology for abiotic stressful environments may lead to increased competitiveness if the transgenes are introgressed into wild populations. Furthermore, the use of regulatory genes such as *DREB* may potentially have a cascading effect on a variety of gene pathways (as compared to the first generation of transgenic crops which were based on one gene-one product systems). Some of these cascade effects will be intended while other will not, some will be known but others will be less easy to define. These present major uncertainties for the approval process and should be intensively addressed through further research (W. De Greef, IPBO, personal communication). This new biosafety framework will, therefore, need to be based on a comprehensive molecular characterization of the expression of regulatory genes and their cascade effects, the very likely different composition of the transgenic plants than their control counterparts under the abiotic stress, the potential impact of such regulatory genes in human health, as well as an environmental impact assessment, which needs to address both the potential escape of the tolerant crop (i.e., increased direct weediness) and the escape of the gene into wild relatives (i.e., increased indirect weediness).

Potential impact of a new paradigm in partnerships to access proprietary transgenic technologies for sub-Saharan African resource-poor farmers

The private, multi-national corporations (MNC hereafter) involved in the seed-biotechnology sector claims that within a decade the first transgenic crops with drought tolerance will be available to farmers. MNC are investing heavily in gene discovery and utilization programs for crop betterment aiming at drought-prone environments to an extent that dwarfs public sector investments in this area. For globally important commodities such as maize, rice or wheat the novel added-value of most public sector investments in gene discovery for drought stress, and transformation may be more important than ever because the intellectual property owners of this MNC technology have often a market interest in crops such as maize and wheat even in the developing world.

It has proven extremely difficult, therefore, to differentiate the public versus private targets, most importantly perhaps, as development investors would like to encourage so-called 'subsistence' farmers to enter the market economy as an effective mechanism of moving out of poverty. Moreover, the liability issues of public sector deployment of proprietary transgenes have rarely been satisfactorily resolved. In this regard, the *DREB* technology which is being licensed for use by CGIAR centers, or the new public research advances such as transgenic maize that express *E. coli*'s glutamate dehydrogenase (*gdhA*) gene (Lightfoot et al. 2007), are important public domain sources of variation for crop betterment in stressful environments. Indeed as pointed out by Cohen (2005), poor nations of the developing world rely on international and national institutional transgenic-crop public research, which should be demand-driven and relevant to end-user needs.

The gene discovery and product development pipeline for commercial transgenic crops for drought-prone environments not surprisingly employs a heavy selection pressure for maximum effect under high input cropping systems where other abiotic and biotic stresses have been largely eliminated, creating a very homogeneous cropping environment. At the present time very little information seems to be available on epistasis and genotype-by-environment interactions associated with transgenes aiming at drought-prone environments when deployed in locally adapted cultivars and grown in complex cropping systems including diverse simultaneous constraints. However, past experience from conventional breeding of complex traits for these target environments suggests that our ability to effectively manipulate these interaction effects will be highly important.

Considering the practical challenges described above, we believe that philanthropic access to a leading proprietary technology from a MNC may not provide the best biological event for CIMMYT stakeholders in resource-poor cropping systems nor provide the best legal environment for rapid product development and deployment. For these reasons CIMMYT proposes a user-led philanthropy-private-public partnership paradigm for the development and deployment of transgenic solutions for maize improvement in the drought-prone environments of sub-Saharan Africa (Fig. 7). Clearly, a detailed full-scale proposal in this area can not be developed until implicit intellectual property management issues are resolved through direct dialogue with relevant MNC. However, once there is conceptual support for this approach from development investors, CIMMYT would propose to initiate a facilitated dialogue with the relevant leading MNC to ensure this transgenic technology becomes available to the resource-poor maize farmers of sub-Saharan Africa. If this new partnership paradigm succeeds, the access to proprietary technologies that can lead to stable grain yields in complex drought-prone areas will allow resource-poor African maize farmers to harvest a reasonable crop in most years, which will almost certainly lead to improved food security, better well-being, enhanced livelihoods and increased opportunities to enter the market economy, even for farmers residing in harsh environments.

Acknowledgements

References

Agarwal P, Agarwal PK, Nair S, Sopory SK and Reddy MK. 2007. Stress-inducible *DREB2A* transcription factor from *Pennisetum glaucum* is a phosphoprotein and its phosphorylation negatively regulates its DNA-binding activity. Molecular Genetics and Genomics 277:189−198.

Agarwal PK, Agarwal P, Reddy MK and Sopory SK. 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants Plant Cell Reports 25:1263−1274.

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Bänziger M and Araus JL. 2007. Recent advances in breeding maize for drought and salinity stress tolerance. *in* Molecular Breeding Towards Salinity and Drought Tolerance (Jenks MA, Hasegawa PM and Jain SM eds.). Dordretch, Netherlands: Springer. In press.

Bänziger M, Setimela PS, Hodson D and Vivek B. 2006. Breeding for improved drought tolerance in maize adapted to southern Africa. Agricultural Water Management 80:212–224.

Bhatnagar-Mathur PB, Devi MJ, Serraj R, Yamaguchi-Shinozaki K, Vadez V and Sharma KK. 2004. Evaluation of transgenic groundnut lines under water limited conditions. International *Arachis* Newsletter 24:33−34.

Bhatnagar-Mathur PB, Devi MJ, Srinivas Reddy D, Lavanya M, Vadez V, Serraj R, Yamaguchi-Shinozaki K and Sharma KK. 2007. Strees-inducible expression of *At* DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. Plant Cell Report DOI 10.1007/s00299-007-0406-8

Behnam B, Kikuchi A, Celebi-Toprak F, Kasuga M., Yamaguchi-Shinozaki K and Watanabe KN. 2007. *Arabidopsis rd29A: DREB1A* enhances freezing tolerance in transgenic potato. Plant Cell Reports 26:1275–1282.

Behnam B, Kikuchi A, Celebi-Toprak F, Yamanaka S,Kasuga M, Yamaguchi-Shinozaki K and Watanabe KN. 2006. The *Arabidopsis DREB1A* gene driven by the stress-inducible *rd29A promoter* increases salt-stress tolerance in proportion to its copy number in tetrasomic tetraploid potato (*Solanum tuberosum*). Plant Biotechnology 23:169–177.

Bennett J. 2003. Opportunities for increasing water productivity of CGIAR crops through plant breeding and molecular biology. Pages 103−126 *in* Water Productivity for Agriculture: Limits and Opportunities from Improvement (Kijne JW, Barker R and Molden D eds). Oxon, Wallingford, UK, CAB International.

Bernier J, Kumar A, Venuprasad R, Spaner D and Atlin G (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Science 47:507–516.

15

Bohnert HJ, Gong Q, Li P and Ma S. 2006. Unraveling abiotic stress tolerance mechanisms – getting genomics going. Current Opinion in Plant Biology 9:180–188.

Bouman BAM. 2007. A conceptual framework for the improvement of crop water productivity at different spatial scales. Agricultural Systems 93:43–60.

Chen M, Wang Q-Y, Cheng X-G, Xu Z-S, Li L-C, Ye X-G, Xia L-Q and Ma Y-Z. 2007. *GmDREB2*, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. Biochemistry and Biophysics Research Communications 353:299–305

Chen WJ and Zhu T. 2004. Networks of transcription factors with roles in environmental stress response. Trends in Plant Science 9:591–596.

CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo). 2005. CIMMYT Business Plan 2006-2010 – Translating the Vision of Seeds of Innovation into a Vibrant Work Plan. CIMMYT, El Batan, Mexico. 42 pp. http://www.cimmyt.org/english/docs/mtp/bp06_10.pdf

CIMMYT. 2007. Translating Strategic Vision to a Vibrant Work Plan – CIMMYT 2008-2010 Medium Term Plan.

Campos H, Cooper M, Habben JE, Edmeades GO and Schussler JR. 2004. Improving drought tolerance in maize: a view from industry. Field Crops Research 90:19–34.

Cohen JI. 2005. Poorer nations turn to publicly developed GM crops. Nature Biotechnology 23:27–33.

De Greef W. 2004. The Cartagena Protocol and the future of agbiotech. Nature Biotechnology 22:811–812.

16

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K and Yamaguchi-Shinozaki K. 2003. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. The Plant Journal 33: 751−763.

Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM and Thomashow MF. 1998. Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. The Plant Journal 16:433–442.

Falkenmark M and Rockström J. 2004. Balancing Water for Humans and Nature: The New Approach in Ecohydrology. London, UK, Earthscan.

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K and Yamaguchi-Shinozaki K. 2006. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. Proceedings of the National Academy of Sciences (USA) 103:1988−1993.

Himmelbach A, Yang Y and Grill E. 2003. Relay and control of abscisic acid signaling. Current Opinion in Plant Biology 6:470–479.

Hoisington D and Ortiz R. 2006. Experience with monitoring and GM-crops in CIMMYT. Pages 106-113 *in* Genetically Modified Organisms in Crop Production and their Effects on the Environment: Methodologies for Monitoring and the Way Ahead. (Ghosh K and Jepson PC eds) Rome, Italy, Food and Agriculture Organization of the United Nations.

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q and Xiong L. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proceedings of the National Academy of Sciences (USA) 103:12987−12992.

17

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K and Yamaguchi-Shinozaki K. 2006. Functional Analysis of Rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant and Cell Physiology 47:141−153.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki Y and Shinozaki K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnology 17:287−291.

Kirigwi FM, Van Ginkel M, Brown-Guedira G, Gill BS, Paulsen GM and Fritz AK. 2007. Markers associated with a QTL for grain yield in wheat under drought. Molecular Breeding DOI 10.1007/s11032-007-9100-3.

Latini A, Rasi C, Sperandei M, Cantale C, Iannetta M, Dettori M, Ammar K and Galeffi P. 2007.Identification of a DREB-related gene in *Triticum durum* and its expression under water stress conditions. Annals of Applied Biology 150:187−195.

Li X-P, Tian A-G, Luo G-Z, Gong Z-Z, Zhnag J-S and Chen S-Y. 2005. Soybean DRE-binding transcription factors that are responsive to abiotic stresses. Theoretical and Applied Genetics 110:1355−1362.

Lightfoot DA, Mungur R, Ameziane R, Nolte S, Long L, Bernhard K, Colter A, Jones K, Iqbal MJ, Varsa E and Young B. 2007. Improved drought tolerance of transgenic *Zea mays* plants that express the glutamate dehydrogenase gene (*gdhA*) of *E. coli*. Euphytica 156:103–116.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K and Shinozaki K. 1998. 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: 1391−1406.

18

Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, and Shinozaki K. 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences (USA) 93:765−769.

Molden AD. 2007. Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture. London: Earthscan − Colombo: International Water Management Institute.

Morris M, Dreher K, Ribaut J-M and Khairallah M. 2003. Money matters (II): costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Molecular Breeding 11:235–247.

Mukhopadhyay A, Vij S and Tyagi AK. 2004. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proceedings of the National Academy of Sciences (USA) 101:6309−6314.

Nakashima K, Tran LP, Nguyen DV, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K and Yamaguchi-Shinozaki K. 2007. Functional analysis of a NAC-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. The Plant Journal 51:617-630.

Nakashima K and Yamaguchi-Shinozaki K. 2005. Molecular studies on stress-responsive gene expression in *Arabidopsis* and improvement of stress tolerance in crop plants by regulon biotechnology. JARQ 39:221–229.

Parry MAJ, Flexas J and Medrano H. 2005. Prospects for crop production under drought: research priorities and future directions. Annals of Applied Biology 147:211–226.

Passioura JB. 1977. Grain yield harvest index and water use of wheat. Journal of Australian Institute of Agricultural Sciences 43:117−120.

Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K and Hoisington D. 2004. Stress-induced expression in wheat of the *Arabidopsis thaliana DREB1A* gene delays water stress symptoms under greenhouse conditions. Genome 47: 493−500.

Pimentel D, Houser J, Preiss E, White O, Fang H, Mesnick L, Barsky T, Tariche S, Schreck J and Alpert S. 1997. Water resources: agriculture, the environment, and society. BioScience 47:97−106.

Qin F, Kakimoto M, Skuma Y, Maruyama K, Osakabe Y, Tran L-SP, Shinozaki K and Yamagushi-Khinozaki K. 2007a. Regulation and functional analysis of *ZmDREB2A* in response to drought and heat stresses in *Zea mays* L. The Plant Journal 50:54-69.

Qin Q, Liu J, Zhang Z, Peng R, Xiong A, Yao Q and Chen J. 2007b. Isolation, optimization and functional analysis of the cDNA enconding transcription factor OsDREB1B in *Oriza sativa* L. Molecular Breeding 19:329−340.

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K and Yamaguchi-Shinozaki K. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA get-blot analyses. Plant Physiology 133:1755−1767.

Reynolds MP and Borlaug NE 2006. Applying innovations and new technologies for international collaborative wheat improvement. Journal of Agricultural Sciences (Cambridge) 144: 95−110.

Reynolds MP, Mujeeb-Kazi A and Sawkins M. 2007. Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments. Annals of Applied Biology 146:239−259.

Ribaut J-M, Hoisington DH, Deutsch JA, Jiang C and Gonzalez-de-Leon D. 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theoretical and Applied Genetics 92:905−914.

Ribaut J-M, Hoisington DH, Deutsch JA, Jiang C and Gonzalez-de-Leon D. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theoretical and Applied Genet 94:887–896.

Ribaut J-M and Ragot M. 2007. Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany 58:351–360.

Rijsberman, F.R. 2006. Water scarcity: Fact or fiction? Agricultural Water Management 80:5–22.

Sakuma Y, Maruyama K, Qin F, Osakabe Y, Shinozaki K, and Yamaguchi-Shinozaki K. 2006. Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. Proceedings of the National Academy of Sciences (USA) 103:18822–18827.

Seki M, Kamei A, Yamaguchi-Shinozaki K and Shinozaki K. 2003. Molecular responses to drought, salinity and frost: common and different paths for plant protection. Current Opinion Biotechnology 14:194–199.

Shen YG, Zhang WK, He SH, Zhang JS, Liu Q and Chen SY. 2003 An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. Theoretical and Applied Genetics 106:923–930.

Stockinger EJ, Gilmour SJ, Thomashow MF. 1997. *Arabidopsis thaliana CBF1* encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proceedings of the National Academy of Sciences (USA) 94:1035–1040.

Tester M, Bacic M. 2005. Abiotic stress tolerance in grasses. from model plants to crop plants. Plant Physiology 137:791–793.

21

Tuberosa R, Salvi S, Sanguineti MC, Landi P, Maccaferri M and Conti S. 2002. Mapping QTLs regulating morphophysiological traits and yield in drought-stressed maize: case studies, shortcomings and perspectives. Annals of Botany 89:941–963.

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K and Yamaguchi-Shinozaki K. 2000. *Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proceedings of the National Academy of Sciences (USA) 97:11632–11637.

Xiong L, Lee H, Ishitani M, Tanaka Y, Stevenson B, Koiwa H, Bressan RA, Hasegawa PM and Zhu J. 2002. Repression of stress-responsive genes by FIERY2, a novel transcriptional regulator in *Arabidopsis*. . Proceedings of the National Academy of Sciences (USA) 99:10899–10904.

Xue GP and Loveridge CW. 2004. *HvDRF1* is involved in abscisic acid-mediated gene regulation in barley and produces two forms of AP2 transcriptional activators, interacting preferably with a CT-rich element. The Plant Journal 37:326–339.

Yamaguchi-Shinozaki, K and Maruyama K. 2004. Monitoring expression profiles of rice genes under cold, drought and high-salinity stresses, and ABA application using both cDNA microarray and RNA gel blot analyses. Pages 6–7 *in* JIRCAS Research Highlights 2003. Tsukuba, Japan, Japan International Research Center for Agricultural Sciences.

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

Yamaguchi-Shinozaki K and Shinozaki K. 2004. Improving drought and cold stress tolerance in transgenic rice. Pages 94–97 *in* Proceedings of World Rice Research Conference "Rice is life: scientific perspectives for the 21st century" Tsukuba, Japan, 5-7 November 2004.

22

Yamaguchi-Shinozaki K and Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and

tolerance to dehydration and cold stresses. Annual Review of Plant Biology 57:781–803.

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Figure 1. Ear harvest of newly bred-maize germplasm (right) and local cultivar (left) in drought-prone environment of Southern Africa

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Figure 2. Selection of re-synthesized wheat under managed drought stress at CIMMYT breeding site in northwest Mexico

Figure 3. Phenotypes of the *DREB1A* line -event 8424 (left) and control plants (right) 15 days after water stress and one watering (8 h) [top photo]. Root phenotype of event 8424 (left) and control (right) [bottom photo] [After: Pellegrineschi et al. 2004]

Figure 4. Field trial of transgenic wheat at CIMMYT biosafety greenhouse (El Batan, Mexico) under realistic agronomic conditions

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Figure 5. Transgenic wheat with *DREB1A* gene showing lower canopy temperature than control under contained field trials mimicking unpredictable mid-season drought [Photos: courtesy of A. Pellegrineschi and M. Reynolds, CIMMYT, El Batan, Mexico]
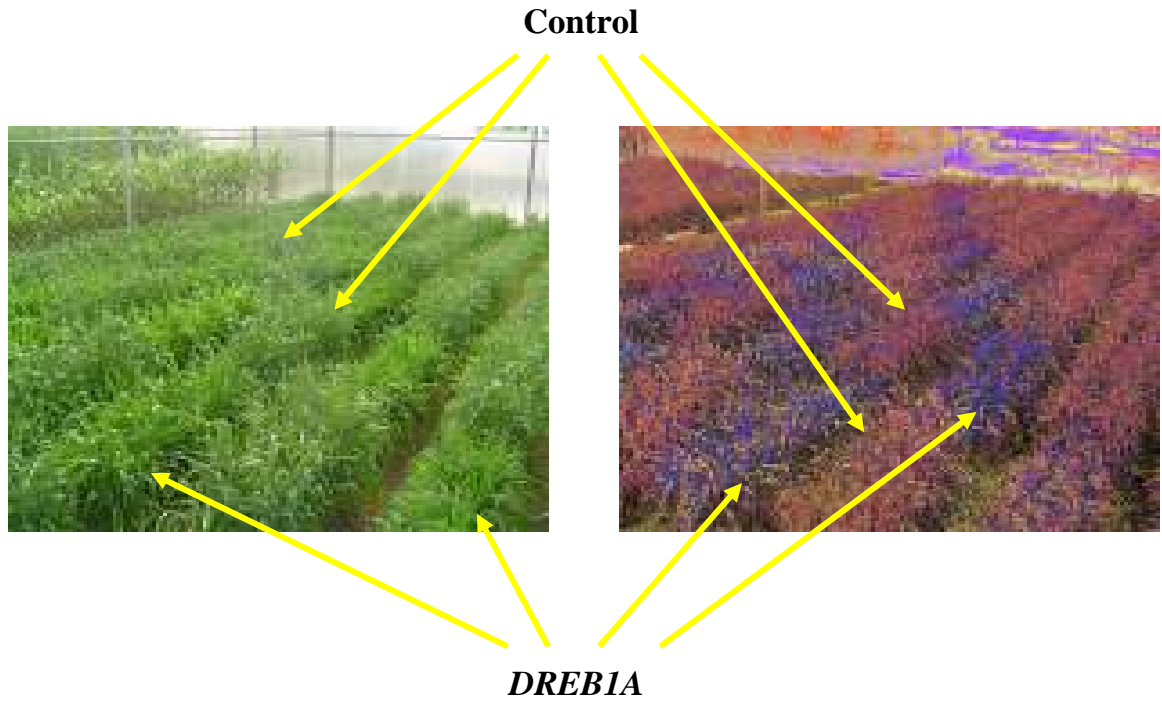

Control

DREB1A

Figure 6. *DREB1A* constructs kindly provide by Dr. Kazuko Yamaguchi-Shinozaki (JIRCAS) to CIMMYT. The graph shows the function of the target genes of *DREB1A*
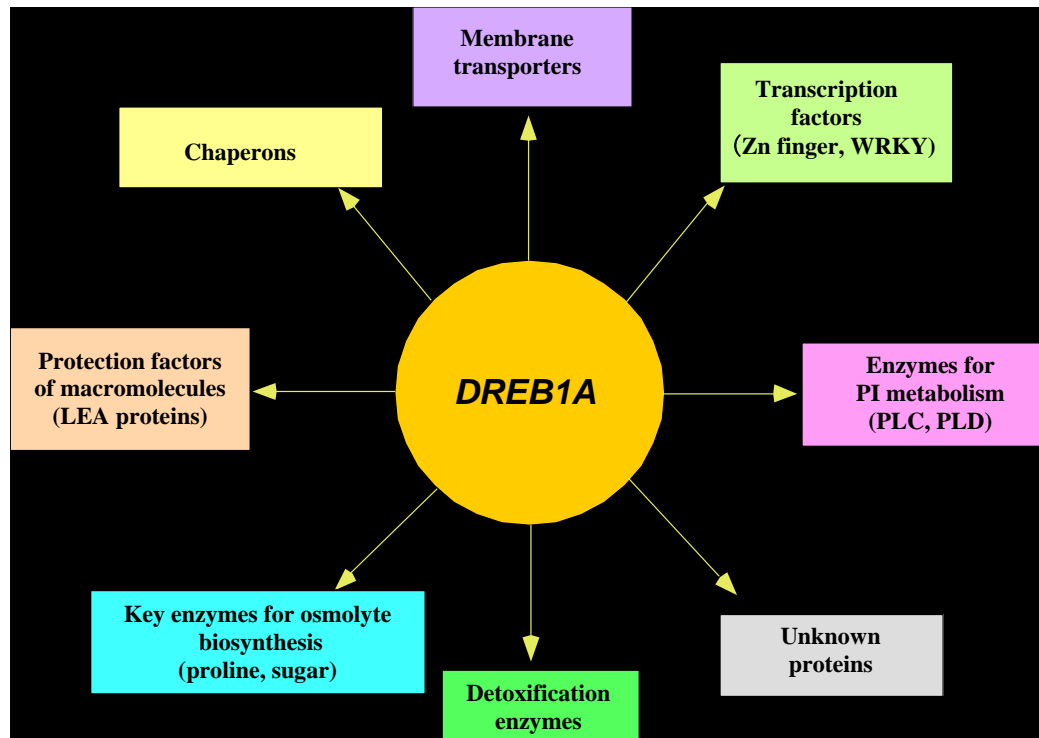
Figure 7. A pro-poor private-public-philanthropic partnership