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Agricultural biotechnology for crop improvement in a variable climate: hope or hype?

Rajeev K. Varshney[1,2,3*], **Kailash C. Bansal**[4,5*], **Pramod K. Aggarwal**[6,7], **Swapan K. Datta**[8] and **Peter Q. Craufurd**[1]

[1] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, India
[2] CGIAR Generation Challenge Programme, c/o CIMMYT, Int APDO Postal 6-641, 06600 Mexico DF, Mexico
[3] School of Plant Biology (M084), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[4] National Research Centre on Plant Biotechnology (NRCPB), IARI Campus, New Delhi 110 012, India
[5] National Bureau of Plant Genetic Resources (NBPGR), New Delhi 110 012, India
[6] Division of Environmental Sciences, NRL Building, Indian Agricultural Research Institute (IARI), New Delhi 110 012, India
[7] CGIAR Challenge Program on Climate Change, Agriculture, and Food Security (CCAFS), International Water Management Institute (IWMI), NASC Complex, Dev Prakash Shastri Marg, Pusa, New Delhi110 012, India
[8] Division of Crop Science, Indian Council of Agricultural Research (ICAR), Krishi Bhavan, Dr. Rajendra Prasad Road, New Delhi 110 114, India

Developing crops that are better adapted to abiotic stresses is important for food production in many parts of the world today. Anticipated changes in climate and its variability, particularly extreme temperatures and changes in rainfall, are expected to make crop improvement even more crucial for food production. Here, we review two key biotechnology approaches, molecular breeding and genetic engineering, and their integration with conventional breeding to develop crops that are more tolerant of abiotic stresses. In addition to a multidisciplinary approach, we also examine some constraints that need to be overcome to realize the full potential of agricultural biotechnology for sustainable crop production to meet the demands of a projected world population of nine billion in 2050.

Why is crop improvement necessary?

The intensification of agriculture in many parts of the world over the past five decades, supported by appropriate research, institutions and policies, has led to an increase in global food grain production from approximately 850 million tons in 1960 to 2350 million tons in 2007 [1]. Although global food production has kept pace with population growth over the past 40 years, almost a billion people, most of who live in the developing world, remain undernourished [2]. It has been projected that global food production must increase by 70% by 2050 to meet the demand caused by this growing global population, increasing incomes and consumption. Food insecurity has also increased in recent times in several regions of the world owing to competing claims for land, water, labor, energy and capital, which is leading to more pressure to improve production per unit of land [1]. Global climate change is

Glossary

Allele mining: the identification and isolation of novel allelic variants associated with the phenotype of interest that exists within large germplasm collections.
Aquaporins: integral proteins of the cell membrane that are required for regulating the movement of water in and out of the cell while excluding ions and metabolites.
Association mapping or linkage disequilibrium (LD) mapping: mapping methods that rely on the historical recombination and nonrandom association of alleles or LD that persists in random mating populations. Association genetics facilitates the identification of marker–trait association based on whole-genome or candidate gene-based LD analysis.
Best linear unbiased prediction: statistical method to calculate random effects in linear mixed models that is widely used for estimating breeding values in plant breeding.
Candidate genes: genes that might be related to the trait of interest. Candidates genes can be identified through either map-based methods such as QTL analysis (positional candidate genes) or functional genomics approaches such as transcriptomics and expression genetics (functional candidate genes).
Cis-genics: GE approach that relies on the identification and transfer of natural indigenous genes or cis-genes, isolated from the same species of the plant or other sexually compatible species.
Cold shock proteins (CSPs): family of proteins that are induced by a decrease in temperature. During cold shocks, most of the cellular protein synthesis processes slow down; however, the number of protein synthesis processes operating in CSPs increases to a maximum during the 'acclimation'.
Constitutive expression: the continuous expression of genes (i.e. no control over expression).
C-repeat binding factors (CBFs): also known as dehydration responsive element binding factors. They are activated by cold stress and have a conserved 'CCGAC' core sequence, which is found in the promoter of many cold-inducible genes.
Food insecurity: the lack of access to an adequate food supply, leading to a deficient food supply at the household level and malnourishment at the individual level.
Gene pyramiding: the process of introducing desirable genes into a single genotype from different donor sources. This is also known as gene stacking.

Corresponding author: Varshney, R.K. (r.k.varshney@cgiar.org)
* These authors contributed equally to this work.

Genetic engineering (GE): the manipulation of the genetic material of an organism using recombinant DNA technology.

Genetic enhancement: the broadening of the genetic base of a species using breeding and/or GE methods.

Genome-wide selection (GWS) or genomic selection (GS): works at the whole genome level without the need for the identification of a subset of markers associated with the traits as in the case of MAS, MABC and MARS. GWS relies on the fact that the genomic regions containing the same rare haplotypes are usually identical by descent, harboring the same QTL allele, and thereby these markers or marker haplotypes, which are in close LD with QTL, can be used for selection.

Genomic estimated breeding values (GEBVs): estimates of the breeding values of genotyped individuals (breeding population), calculated based on marker effects derived from the genotyping and phenotyping data obtained from trained individuals (training population).

Intergovernmental Panel on Climate Change (IPCC): scientific and intergovernmental organization, developed as a collaborative effort of the UN Environment Programme and the World Meteorological Organization. The aim of the IPCC is to review the scientific, technical and socioeconomic impacts of climate change.

Marker-assisted backcrossing (MABC): marker-aided foreground selection to introgress precisely the donor segment into the elite breeding line accompanied by marker-assisted background selection to ensure the maximum recovery of recurrent parent genome.

Marker-assisted recurrent selection (MARS): marker-aided population improvement scheme relying on the recovery of superior or ideal genotypes, which are generally made up of various genomic fragments harboring smaller effect QTLs. The isolation of such an ideal genotype is not possible in simple biparental mapping populations.

Molecular breeding (MB): the process of genetic improvement through the deployment of molecular tools such as DNA markers in traditional breeding. MB enhances genetic gain by increasing the selection efficiency coupled with the reduced length of breeding cycles.

Next-generation sequencing (NGS) technologies: high-throughput sequencing technologies such as Roche/454 (http://www.454.com/), Solexa/Illumina (http://www.illumina.com/) and AB-SOLiD (http://www.appliedbiosystems.com/), which provide reduced cost per data point. NGS techniques are ideal for resequencing genomes, but currently these are being used for *de novo* whole-genome sequencing in many crops.

Nitrogen use efficiency (NUE): expressed in terms of grain yield per unit of available soil nitrogen. NUE can be divided into two components: uptake efficiency (to take nitrogen from the soils) and usage efficiency (to convert the nitrogen uptake into protein).

Quantitative trait loci (QTLs): genomic regions associated with complex quantitative traits governed by several large effect as well as smaller effect genes. Special statistical software is needed to identify the locations and effects associated with these regions.

RNA chaperones: class of proteins required for the proper folding of RNA or for resolving incorrectly folded RNA structures.

RNAi: the natural mechanism of silencing the expression of genes with the help of RNA molecules such as miRNA and siRNA. RNAi facilitates the rapid identification of gene functions.

Targeted gene replacement: the *in vitro* modification of a cloned DNA fragment and subsequent introduction into the host cell through homologous recombination or gene targeting.

Training population: one of the components of a GS scheme. Genotyping and phenotyping data are recorded for 'model' or 'trained' individuals, which are subsequently used to calculate the GEBVs of individuals.

Transgenic or genetically modified organism: contains a foreign gene that has been introduced into its genome by GE.

Wild relatives: wild species, particularly those closest to domesticated plants that might harbor lots of novel variations not available in the cultivated germplasm pool.

likely to increase the problems of food insecurity, hunger and malnutrition for millions of people, particularly in south Asia, sub-Saharan Africa and small islands [3–5], and also further aggravate the current trends in land degradation, especially in semi-arid tropical regions [6].

Whereas global temperatures are predicted to increase by 2.5–4.3 °C by the end of the century [3], with significant effects on food production [4,5,7,8] and malnutrition [5], it is also evident that agriculture is currently affected by increasing climate variability, especially temperature [9,10]. For example, it has been estimated that rising global temperatures between 1981 and 2002 reduced the yields of major cereals by $5 billion per year [9]. Heat waves and drought in Europe in 2003 [11] significantly reduced productivity [e.g. maize (*Zea mays*) yield in northern Italy by 36%]. In 2009/2010, heat waves also affected wheat (*Triticum aestivum*) production in central Asia, and extreme flooding affected agricultural production in south Asia. In addition to the challenge of temperature extremes (hot and cold) and drought or water stress as well as flooding associated with climatic variability, the incidence and severity of biotic stresses such as pests, diseases and the invasion of alien weed species are also likely to be greater. Cropping systems at greatest risk include wheat and rice (*Oryza sativa*) in south and southeast Asia and maize in southern Africa [8,12–14].

In the context of current climate variability, as well as predicted increases in mean temperature and annual precipitation, what do recent advances in agricultural biotechnology offer the genetic enhancement of agricultural crops so that they are better adapted to biotic and abiotic stresses, leading to higher crop productivity? In this review, we critically examine the role that agricultural biotechnology could play in addressing biotic and abiotic constraints to greater food productivity.

Biotechnological interventions

The objective of plant breeding for stress environments is to accumulate favorable alleles that contribute to stress tolerance in a plant genome. Genes that confer stress resistance can be sourced from germplasm collections, including wild relatives of crops that are held in genebanks or organisms that currently live in the habitats of water deficit or excess, extreme temperature and salinity that have evolved to cope with those conditions [15]. Although some progress has been made through conventional breeding [16], breeding for abiotic stress tolerance is constrained: (i) by the complex nature of abiotic stress tolerance (timing, duration, intensity, frequency) and thereby its quantification and repeatability; (ii) because undesirable genes are also transferred along with desirable traits; and (iii) because reproductive barriers limit the transfer of favorable alleles from diverse genetic resources.

Biotechnology is a viable option for developing genotypes that can perform better under harsh environmental conditions, particularly for (ii) and (iii) above. For instance, advances in genomics coupled with bioinformatics and stress biology can provide useful genes or alleles for conferring stress tolerance.

Superior genes or alleles where they have been identified in the same species can be transferred into elite genotypes through molecular breeding (MB). Moreover, by using an approach such as genetic engineering (GE), there is no barrier to transferring useful genes or alleles across different species from the animal or plant kingdoms. As a result, biotechnology approaches offer novel strategies
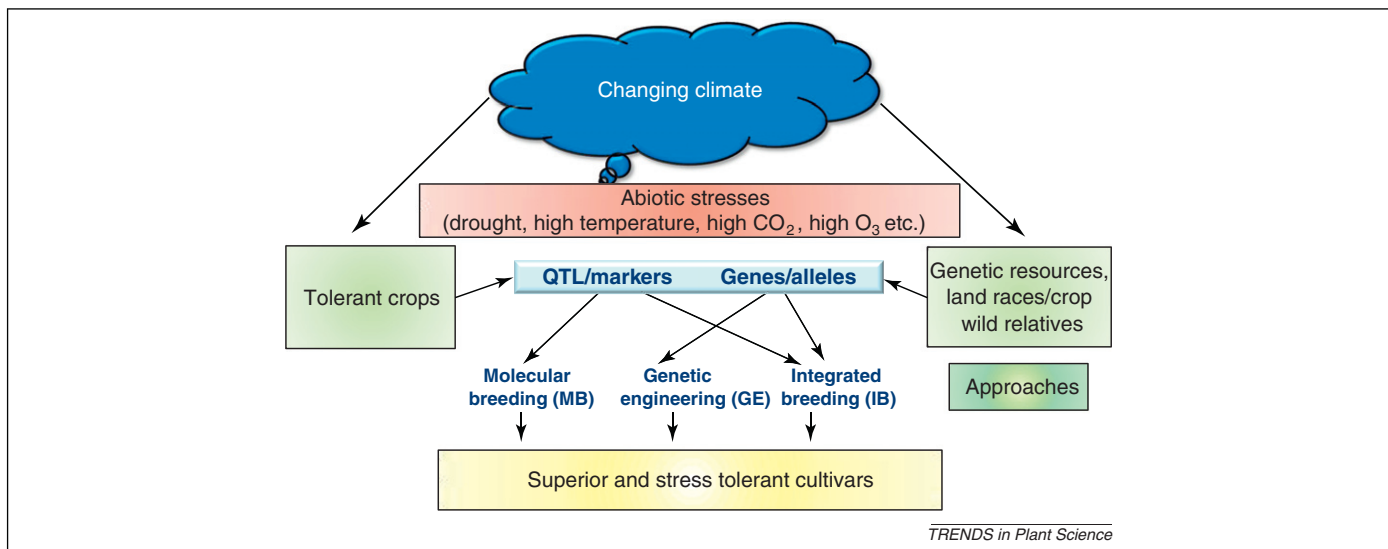
Figure 1. An integrated approach to developing crops that are better adapted to abiotic stresses. Germplasm collections including tolerant crops, landraces and wild relatives of crops can be used to identify or isolate QTL(s), gene(s) or allele(s) that confer tolerance to abiotic stresses such as drought and high temperature by using modern genomics approaches. Although candidate QTL(s) can be deployed through MB approaches such as MABC, MARS and GS, the most promising candidate genes along with appropriate promoters can be used by using a GE approach in conventional breeding programs. It is anticipated that the use of an integrated approach, as suggested here, should facilitate the development of designer crops that are better adapted to abiotic stresses and thereby better able to tolerate future climate variability.

for producing suitable crop genotypes that are able to resist drought, high temperature, submergence and salinity stresses (Figure 1). Key strategies where genetic enhancement for abiotic stress tolerance has led to crop improvement are outlined in Box 1.

Several key approaches for improved crop productivity in an environment with high temperatures and high CO_2 have been discussed in recent reviews [17,18]. Similarly, Ainsworth and colleagues critically analyzed the biotechnological approaches that could be used to develop crops with potentially improved productivity in an environment with high temperature, high CO_2 and high ozone [19]. These included manipulating leaf photosynthesis, photosynthate partitioning, total biomass production and nitrogen use efficiency (NUE) (see Glossary). Improved NUE in crops should lead to reduced fertilizer application and thereby lower emissions of greenhouse gases into the atmosphere. More than 50% of all US greenhouse gas emissions from agriculture are associated with fertilizer application and other cropping practices [20]. Rather than focusing on individual stresses, the biotechnology community should use biotechnological approaches to tackle multiple stresses directly under field conditions [21]. Our increased understanding of the molecular and genetic bases of abiotic stress responses in plants should enable us to use crop-specific MB, GE and preferably integrated programs to introduce resistance to multiple stresses.

Key agricultural biotechnology approaches: prospects and progress
Molecular breeding
Significant advances have been made in the area of genomics over the past ten years. Genome sequences are available now for many crop species such as rice, [22–24], poplar (*Populus trichocarpa*) [25], sorghum (*Sorghum bicolor*) [26], maize [27] and soybean (*Glycine max*) [28]. Furthermore, the advent of so-called 'next-generation sequencing'

(NGS) technologies have made it possible to sequence the transcriptomes or genomes of any species (and for any number of individuals) relatively quickly and cheaply [29]. As a result, genome sequences have started to become available for less studied crops such as cucumber (*Cucumis*

Box 1. Key biotechnological strategies for improving abiotic stress tolerance

MB approach

 (i) The development of genomic resources such as molecular markers including simple sequence repeats, single nucleotide polymorphisms and marker genotyping platforms.
 (ii) The development of biparental mapping populations by using genetically and phenotypically diverse parental lines or the selection of a natural population representing diversity for abiotic stress tolerance traits.
(iii) The use of linkage mapping or association mapping approaches to identify the QTLs or markers associated with abiotic stress tolerance-related parameters, such as leaf water retention, high rates of leaf photosynthesis, stomatal conductance, osmotic adjustment and faster canopy and root development.
(iv) The validation of the QTLs or markers in a breeding germplasm that have a different genetic background.
 (v) The use of an appropriate MB approach such as MABC, MARS or GWS to develop superior crop genotypes.

GE approach

 (i) The identification of genes encoding signaling proteins, TFs and effector proteins, and novel stress responsive promoters controlling multiple stress tolerance.
 (ii) The identification of genes regulating stomatal opening and closure and stress-induced expression to enhance water use efficiency in crops.
(iii) The genetic transformation and development of elite crop genotypes with tolerance to high temperature stress and other environmental stresses.
(iv) The assessment of promising transgenic lines for multiple stress tolerance under field conditions.
 (v) The deregulation of transgenic lines to enable the release of a superior line or variety.

sativus) [30], pigeonpea (*Cajanus cajan*) (http://www.icrisat.org/gt-bt/IIPG/home.html) and large and complex genome species such as wheat (http://www.genomeweb.com/sequencing/wheat-genome-sequenced-roches-454) and barley (*Hordeum vulgare*) (http://barleygenome.org/). These genome or transcriptome sequences coupled with genetic approaches can be used for identifying suitable genes conferring stress tolerance that can be deployed in crop improvement either by using MB or GE approaches. The use of genome sequences to identify genes associated with drought tolerance can be demonstrated by taking the example in sorghum, a species well adapted to drought-prone regions. Sorghum genome analysis has indicated that the characteristic adaptation of sorghum to drought might be partly related to the expansion of one miRNA and several gene families. Rice miRNA 169 g, upregulated during drought stress [31], has five sorghum homologs (sbi-MIR169c, sbi-MIR169d, sbi-MIR169.p2, sbi-MIR169.p6 and sbi-MIR169.p7). The computationally predicted target of the sbi-MIR169 subfamily comprises members of the plant nuclear factor Y (NF-Y) B transcription factor family, linked to improved performance under drought by *Arabidopsis* (*Arabidopsis thaliana*) and maize [32]. Cytochrome P450 domain-containing genes, often involved in scavenging toxins such as those accumulated in response to stress, were found to be abundant in sorghum (326 cf 228 in rice). Expansins, enzymes that break hydrogen bonds and are responsible for a variety of growth responses that could be linked to the drought tolerance of sorghum, occurred in 82 copies in sorghum cf 58 in rice and 40 each in *Arabidopsis* and poplar [26].

The MB approach involves first identifying quantitative trait loci (QTLs) for traits of interest, such as tolerance to abiotic stresses. Until recently, QTLs were identified by linkage mapping [33], but now association genetics has started to supplement these efforts in several crops [34,35]. Nested association mapping, which combines the advantages of linkage analysis and association mapping in a single unified mapping population, is also being used for the genome-wide dissection of complex traits in maize [36]. Association mapping, compared with linkage mapping, is a high-resolution and relatively less expensive approach. In the near future, it is likely to be routinely used for identifying traits associated with abiotic stresses [34], particularly given the availability of high-throughput marker genotyping platforms [37]. An example of the systematic use of association mapping for drought tolerance is the collaborative project between Cornell University and CIMMYT (http://www.maizegenetics.net/drought-tolerance). After identifying the markers associated with QTLs or genes for traits of interest, the candidate QTLs or genes can be introgressed in elite lines through marker-assisted backcrossing (MABC).

Although MABC has been successful in developing superior genotypes for traits controlled by major effect gene(s) or QTLs, for example bacterial blight and blast resistance in rice [38–48], few examples are available for complex traits such as tolerance to drought and heat, which are the key traits that need to be targeted for developing crops that are adapted to low rainfall and high temperature conditions. However, MB has been success-

fully used in rice, with one major effect QTL each for submergence tolerance [49] and drought tolerance [50] identified and used in this approach.

One of the difficulties of developing superior genotypes for abiotic stresses such as drought or heat is that these traits are generally controlled by small effect QTLs or several epistatic QTLs [51]. MABC does not seem to be an effective approach for introgressing QTLs such as these, especially because of the large sizes of the backcross populations required to pyramid several QTLs in the same genetic background. However, two newer MB approaches –marker-assisted recurrent selection (MARS) and genome-wide selection (GWS) or genomic selection (GS) – can be used to overcome this problem [37,52].

The estimated genetic gain that is feasible using MARS or GWS is greater than can be obtained using MABC for transferring or pyramiding superior QTLs or gene alleles for complex traits such as drought or heat tolerance in one genetic background [53,54]. Although the MARS approach is used routinely in private sector breeding programs [54,55], there are no published reports on the use of MARS in public breeding programs. Another comprehensive approach for improving complex traits is based on GWS. Although MABC and MARS require QTL information for complex traits, information on marker–trait associations is not necessarily required for GWS [56,57]. Basically, GWS deals with the prediction of the genomic-estimated breeding values (GEBVs) of progeny. In this context, there is first a need to have the phenotyping data as well as genome-wide marker profiling on a 'training population'; subsequently, GEBVs can be calculated based on phenotyping and marker datasets. These GEBVs are then used to select the superior progeny lines for advancement in the breeding cycle [57,58]. Several computational tools are available or are being developed to calculate GEBVs, such as the Best Linear Unbiased Prediction method and the geostatistical mixed model [59] (http://genomics.cimmyt.org/#Software). However, at present there is little information available on the use of GWS in crop plants in public sector breeding programs, although some groups have started to explore this approach in crops such as maize (http://genomics.cimmyt.org/, http://www.synbreed.tum.de/index.php?id=31).

Genetic engineering

The upsurge of genomic information and the use of associated computational biology tools over the past decade have led to the identification of signaling pathways and regulatory genes and networks controlling complex traits related to environmental stresses. Crop GE with signaling components and transcription factors (TFs) leads to the expression of their target transcriptome that consists of several genes involved in stress adaptation. For example, the enhanced production of the signaling hormone abscisic acid (ABA) by the overexpression of the *LOS5/ABA3* gene encoding a Molybdenum Cofactor Sulfurase, required for ABA synthesis, conferred enhanced drought tolerance in transgenic rice plants under field conditions [31]. Similarly, the overexpression of the rice *AP37* (an APETALA2-type TF) gene resulted in the enhanced expression of several target genes and produced 16–57% higher grain yield
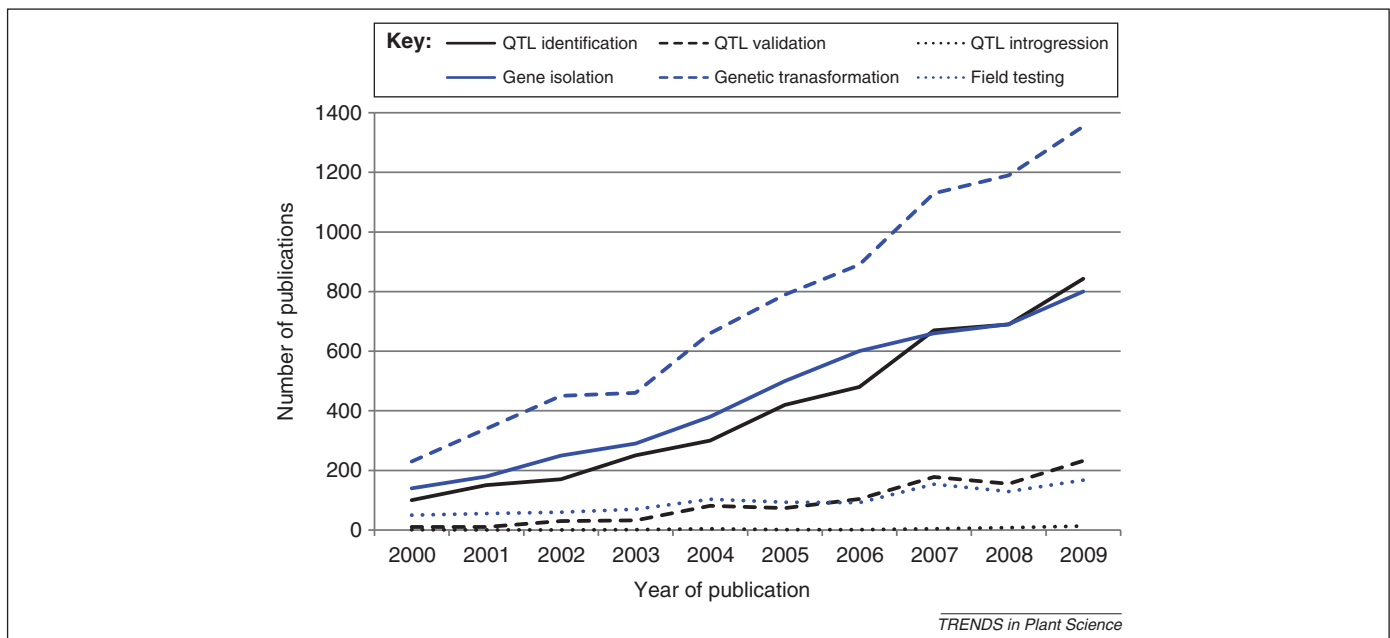
Figure 2. Trends in publications related to MB and GE approaches used to develop crops that are better adapted to abiotic stresses. Although publications on QTL identification and genetic transformation are increasing every year, only a limited number of publications have become available on QTL validation or introgression and the field testing of transgenics. (Source: Google Scholar, Sept 20, 2010).

under field drought stress conditions [60]. Hence, transcriptome engineering seems to be promising for the development of abiotic stress-tolerant crops. However, inducible expression rather than the constitutive overexpression of TFs is preferable owing to the severe growth retardation and reduction in seed production that can occur even under normal environmental conditions in transgenic crops with constitutive expression of TFs [61]. Nonetheless, several transgenic crops have been engineered using C-repeat binding factors (CBFs) and other TFs without a yield penalty [62,63]. Transgenic rice plants overexpressing *Arabidopsis CBF3/DREB1A* or *ABF3* TF showed improved tolerance to drought and salinity without growth retardation [64]. However, only a few crops such as rice [31,60,65], maize [32,66,67] and canola (*Brassica napus*) [66,68], expressing the desired TF and other genes, have been tested under real field stress conditions [63] (Figure 2).

RNA chaperones known for their active role, particularly in mediating transcription and translation both in bacteria and plants, have also been shown to increase yield under multiple stresses. For instance, Monsanto (http://www.monsanto.com/) researchers showed that bacterial cold shock proteins (*Csps*) can confer improved stress adaptation in multiple plant species. For instance, *CspB* codes for and is responsible for an RNA chaperone, which is a commonly occurring protein molecule that binds to RNAs and facilitates their function. The gene was first identified in bacteria subjected to cold stress conditions, and further research has demonstrated that *CspB* helps plants cope with drought stress. In maize and rice, *CspB* works by helping the plant maintain growth and development during times of inadequate water supply [67].

Recently, a gene encoding aquaporin (NtAQP1) was identified in tobacco (*Nicotiana tabacum*) and shown to

provide protection against salinity stress in transgenic tomatoes (*Solanum lycopersicum*) [69]. NtAQP1 plays a key role in preventing root/shoot hydraulic failure, enhancing water use efficiency and thereby improving salt tolerance. It simultaneously increased both water use and photosynthetic efficiency in plants. Moreover, the NtAQP1 gene, which increases stomatal conductance, might also lower canopy temperature and thereby reduce the level of heat stress experienced by plants. By contrast, decreased stomatal conductance and thereby transpiration by the suppression of farnesyltransferase genes (FTA or FTB) by RNAi in transgenic canola resulted in significantly higher yields compared with controls in a 3-year field trial [68,70]. To make up for the water loss owing to higher stomatal conductance in the NtAQP1 transgenic plants, pyramiding genes for osmolyte biosynthesis expressed specifically in roots could lead to the growth of deeper roots, potentially enabling water uptake from deeper soil layers [71].

A combination of genes is required to offset the adverse impact of climate variability on plants. Apart from genes encoding effector proteins, signaling proteins and/or TFs, there is a need for a repertoire of promoters to drive transgene expression in a precise and predetermined fashion in specific tissues or plant organs. Appropriate promoters need to be selected, depending upon the gene used, to obtain desirable transgenic plants with high yield stability under stress conditions. For example, as discussed earlier, the constitutive expression of *ZmNF-YB2* in maize conferred enhanced drought tolerance [32]. By contrast, transgenic rice plants overexpressing *OsNAC10* under the root-specific promoter RCc3, but not under the control of the constitutive GOS2 promoter, conferred a yield advantage under drought stress conditions in the field [32,72,73]. The use of appropriate promoters might enable gene pyramiding through GE to tackle the issue of tolerance to multiple

stresses at different stages of plant growth and development [74].

TFs implicated in more than one type of stress might also be identified. The role of TFs in enhancing agricultural productivity via increased leaf photosynthesis, modified plant architecture and faster plant growth rates has been discussed in several articles [62,75]. Other TFs, such as DREB2A with diverse roles in both biotic and abiotic stresses, could possibly be deployed for engineering multiple stress tolerance along with enhanced yields [63]. However, the key concern about TF transgenics is whether they will perform consistently under drought and/or heat stress conditions in the field.

Integrated biotechnology approach
Although the biotechnology community has remained focused on either MB or GE approaches [44,76], it is evident that there is a need to target complex problems caused by drought and heat by using integrated biotechnology approaches. In this context, the maize community represents an excellent example, undertaking several major projects including Water Efficient Maize in Africa (WEMA, http://www.aatf-africa.org/wema/en/), Drought Tolerant Maize for Africa (DTMA, http://dtma.cimmyt.org/) and Improved Maize for African Soils (IMAS, http://www.cimmyt.org/en/projects/improved-maize-for-african-soils). These projects are using conventional breeding, MB and GE approaches in collaboration with international partners, including multinational companies such as Monsanto (www.monsanto.com/) and Pioneer (http://www.pioneer.com/). Under the WEMA initiative, CIMMYT is providing high-yielding maize cultivars that are adapted to African conditions as well as expertise in conventional breeding and testing for drought tolerance. Monsanto is providing proprietary germplasm, advanced breeding tools and expertise, and drought-tolerant transgenes developed in collaboration with BASF (http://www.basf.com/). The cultivars developed through the initiative will be distributed to African seed companies through the African Agricultural Technology Foundation (AATF) without royalties, and made available to smallholder farmers as part of their seed businesses. For example, over 50 new maize hybrids and open-pollinated maize cultivars have been developed and provided to seed companies and nongovernment organizations for dissemination under the DTMA initiative. These drought-tolerant maize cultivars produce approximately 20–50% higher yields under drought than do other maize cultivars and several of them have already reached farmers' fields. The IMAS initiative, by contrast, is developing maize varieties that are better at capturing the small amount of fertilizer that African farmers can afford and that use the nitrogen they take up more efficiently to produce grain (i.e. to increase NUE).

In addition to MB and GE, some new approaches have recently become available that should be integrated with MB and GE to tackle complex stresses in a concerted manner. These approaches include (i) NGS or transcriptomics and proteomics approaches for isolating novel genes and promoters for multiple abiotic stress tolerance [29]; (ii) gene targeting for the genetic modification of crops [77,78]; (iii) marker-free transgenic crop development [79]; (iv) the development of cis-genics [80]; (v) allele mining for candidate genes in germplasm collections [81]; and (vi) the creation and use of mutations by deploying Targeted Induced Local Lesions in Genomes (TILLING) [82].

Constraints and opportunities for the use of biotechnology approaches
Although several technological advances have been made in the recent past in the field of biotechnology, one of the major challenges is the widening gap between the rate of the development of new technologies and their deployment in applied breeding programs for crop improvement. For instance, many genes for different stresses have been cloned and characterized in models as well as some crop plant species, and in some cases, successful reports on the development of transgenics have also been reported, e.g. rice for *LOS5/ABA3* [31] and *AP37* [60] (Figure 2). However, to date, no reports of a released transgenic variety for drought tolerance have been published, even though *Bt*-transgenic crops have been widely adopted globally [83].

Although several reports are available on the identification or even validation of QTLs or markers for abiotic stress tolerance, their successful deployment in the development of a superior cultivar has had only limited success. Even in the case of rice, there are only two examples where QTLs for abiotic stress tolerance, namely submergence tolerance [49] and drought tolerance [50], have been successful. This limited success of biotechnology for developing abiotic stress-tolerant cultivars indicates one or more of the following points: (i) The nature of abiotic stress is complex with variations in the timing, duration and intensity of stress interacting with different stages of plant development. (ii) Abiotic stress tolerance is often measured using traits, such as yield under stress, that are integrators over time of many processes or mechanisms. Therefore, approaches involving the introgression of one gene or QTL using GE or MB is usually not sufficient to develop drought- or heat-tolerant lines unless that gene or QTL has a large effect on a particular key process (e.g. disease resistance [44,45]. (iii) Our capacity to phenotype is limited by our understanding of abiotic stress tolerance mechanisms, which ultimately limits all conventional or molecular plant breeding efforts. There is also a lack of appropriate and large-scale phenotyping facilities in public research institutes, particularly in developing countries. (iv) The appropriate MB method (i.e. MARS or GWS) needs to be used instead of MABC for achieving higher genetic gain for complex traits. (v) GE requires the identification of appropriate promoters, particularly for gene stacking.

It is now time to use interdisciplinary approaches to tackle the serious challenges of complex abiotic stresses, and the scientific community and science policymakers should consider the following approaches: (i) selection of the most appropriate set of genes or QTLs for either a GE or a MB approach; (ii) emphasis on precise and large-scale phenotyping based on a good understanding of the key processes for drought and heat tolerance either alone or in combination. Field-based facilities such as Temperature Free Air CO_2 Enrichment (or T-FACE) [17,18] will be

increasingly important in this regard because they allow for phenotyping under more natural conditions; (iii) deployment of integrated biotechnology approaches, including appropriate MB and GE methodologies, together with new genomics and conventional breeding [81]; (iv) long-term investments in the public sector to develop the next generation of biotech crops; (v) emphasis on the adoption of biotechnology research in breeding programs [54,84]; (vi) simplifying the process of the biosafety regulation of transgenic crops [84–86] and (vii) creating appropriate public awareness in developing countries about the use of biotechnology approaches.

Lastly, we should not forget that ultimately all products of plant breeding, conventional, MB or GE, have to be delivered to farmers in the form of the seed of improved cultivars. At the present time, many small farmers do not have access to seed of improved cultivars, and in the short-term it is the failure of seed systems as much as the lack of abiotic stress-tolerant cultivars that is the major limitation [1,87].

Future of biotechnology approaches for crop improvement

Biotechnology approaches have the potential to enhance crop production under different stress conditions. On the one hand, abiotic stresses are complex in nature; on the other hand, there are several challenges that have restricted the realization of the full potential of using biotechnology approaches in crop breeding. Nevertheless, with current and fast emerging technologies such as RNAi [88], targeted gene replacement using zinc-finger nucleases, chromosome engineering, MARS and GWS, NGS and nanobiotechnology, the future seems bright with respect to the development of designer crops with improved features that can use natural resources such as water, soil nutrients, atmospheric carbon and nitrogen with a far greater efficiency than ever before. Although MB-derived products have been accepted and adopted, GE-derived crops still have a long way to go to gain universal acceptance and reach farmers' fields. Even though the benefits to small and resource-poor farmers have been demonstrated and the GE technology is becoming more popular, the political will to facilitate this process is weak [84]. Indeed, there have been many calls for the global harmonization of regulations, which would make the requirements compatible and consistent [89]. Regulatory harmonization would help remove artificial trade barriers, expedite the adoption of GE crops, protect developing countries from exploitation and bring the benefits of GE products to the consumer. Eventually, the adoption of biotech crops to mitigate abiotic stresses that are expected to increase in frequency and intensity in coming years will depend on public perceptions and public acceptance, as well as on cultural and institutional processes in developing countries.

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