

Genomics-based approaches to improve drought tolerance of crops

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The genetic bases of the molecular, cellular and developmental responses to drought involve many gene functions regulated by water availability. Genomics-based approaches provide access to agronomically desirable alleles present at quantitative trait loci (QTLs) that affect such responses, thus enabling us to improve the drought tolerance and yield of crops under water-limited conditions more effectively. Marker-assisted selection is already helping breeders improve drought-related traits. Analysis of sequence data and gene products should facilitate the identification and cloning of genes at target QTLs. Based on such premises, we envision a quick broadening of our understanding of the genetic and functional basis of drought tolerance. Novel opportunities will be generated for tailoring new genotypes 'by design'. Harnessing the full potential of genomics-assisted breeding will require a multidisciplinary approach and an integrated knowledge of the molecular and physiological processes influencing tolerance to drought.

Approaching the complexity of crop yield under drought conditions

Of all the abiotic stresses that curtail crop productivity, drought is the most devastating one and the most recalcitrant to breeders' efforts. In the past, breeding efforts to improve drought tolerance have been hindered by its quantitative genetic basis and our poor understanding of the physiological basis of yield in water-limited conditions [1,2]. Further complexity derives from the occurrence of other abiotic stresses that often amplify the negative impact of drought on growth and metabolism [3]. From an application point of view, it is crucially important to select genotypes able to optimize water harvest and water-use efficiency while maximizing yield in relation to the dynamics of the drought episodes prevailing in each target environment [4]. Therefore, in this review, the term 'drought tolerance' relates to final yield rather than to the capacity of the plant to survive in water-limited conditions.

Compared to conventional approaches, genomics offers unprecedented opportunities for dissecting quantitative traits into their single genetic determinants, the so-called quantitative trait loci (QTLs, see Glossary), thus paving the way to marker-assisted selection (MAS) [5,6] and, eventually, cloning of QTLs [7] and their direct

manipulation via genetic engineering. The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops (Table 1) indicates a growing interest in this approach. Furthermore, new genomics platforms, sequencing and bioinformatics (Tables 2 and 3) have all added new dimensions for deciphering and manipulating the genetic basis of drought tolerance [8–10]. However, despite all the recent technological breakthroughs, the overall contribution of genomics-assisted breeding to the release of drought-resilient cultivars has so far been marginal. This review critically analyses how genomics can contribute to accelerating the release of improved, drought-tolerant cultivars. In particular, we will address how the information on QTLs governing the response to drought and candidate genes responsible for QTL effects can be used to elucidate the physiological basis of drought tolerance and to select genotypes with an improved yield under water-limited conditions.

QTLs: the unavoidable crossroad towards drought tolerance?

Given that the morpho-physiological traits that affect the tolerance of crops to drought are quantitatively inherited [2], the discovery of QTLs plays a central role in their improvement through MAS. Previous reviews have discussed the merits and limitations of a myriad of traits acting from the cellular to the whole crop level [2,11,12]. Among this seemingly endless list of morpho-physiological attributes, we will mainly focus on root traits because of the crucial role of roots in harvesting water from the soil and the difficulty of investigating and selecting for root traits using conventional approaches (Box 1). Roots show a high degree of developmental plasticity, particularly in response to water and nutrients [13]. Although this plasticity is under genetic control to a varying degree [14] and several QTLs for root traits have been identified in rice [15] and maize [16,17], no study has so far addressed the identification of QTLs for the response of root elongation rate to soil moisture. However, recent work in maize has identified QTLs for the response of leaf elongation rate to soil moisture, temperature and evaporative demand [18]. Remarkably, a model based on the combined effects of the major QTLs was able to predict 74% of the variability for leaf elongation rate measured among recombinant inbred lines of the mapping population [18]. Applying this modelling approach to root elongation rate should provide valuable insight into the role of root plasticity in the 'Genotype × Environment' (G × E) interaction under different water regimes and allow MAS to be used

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Glossary

BAC (bacterial artificial chromosome): a plasmid in which large inserts of up to 150 kb of DNA can be inserted.

Candidate gene: a gene that based on its putative function and map position can account for a given phenotype.

EcoTILLING: the application of the TILLING technique to search natural allelic variation at a target gene or sequence. It enables the recognition of allelic variants differing for 1 bp out of ~1000 bp.

eQTL (expression Quantitative Trait Locus): a genetic locus where allelic variation affects the level of gene expression. The expression is most often quantified in terms of the amount of mRNA in a microarray-based analysis, but the same principle has been applied to genetic control of the protein level.

Genotype \times Environment (G \times E) interaction: a condition where the relative differences among phenotypes and/or their ranking vary according to environmental conditions (e.g. water availability).

Haplotype: a combination of alleles at different loci on the same chromosome segment.

Ideotype breeding: breeding activity aimed at producing new genotypes with novel morpho-physiological features that fit a pre-defined architecture thought to be advantageous based on experimental physiology and/or modelling.

Isogenization: a combination of genetic procedures aimed at producing lines (genotypes) differing for the allelic constitution at a single gene or QTL. The genetic effect of a QTL can be more easily estimated by contrasts among isogenic lines because of the lack of the portion of phenotypic variance generated by the segregation of other QTLs influencing the trait.

LD (linkage disequilibrium): the level of non-random assortment of alleles at different loci.

Marker-assisted selection: a selection method based on the individual genotypes at segregating marker loci linked to genes or QTLs controlling the target trait. It is particularly valuable for improving traits with low heritability or that are difficult or expensive to evaluate.

Metabolomics: the application of platforms able to identify and measure metabolites.

Osmotic adjustment: the process of water potential reduction of a cell, without a decrease in turgor, obtained by the coordinated accumulation of ions in the vacuole and compatible solutes (e.g. molecules not interfering with enzymatic functions such as proline, sorbitol and/or glycine betaine) in the cytoplasm. A cell can absorb water as long as its water potential is below that of the water source.

Proteomics: the application of platforms able to identify and measure a large collection of proteins.

Pyramiding of genes and/or QTLs: the process of merging desirable alleles of target genes and/or QTLs into one genetic background. It is usually implemented by marker-assisted selection.

QTL \times Environment interaction: a condition where the relative effect of a QTL on the overall genetic variability varies according to environmental conditions.

Quantitative trait: a trait whose phenotypic value is influenced by multiple genes and environmental conditions. For such traits, individuals are measured rather than classified.

Quantitative trait locus (QTL): a chromosome region whose allelic variation can be statistically associated with the value of a quantitative trait across mapping populations and/or suitable panels of accessions characterized by the presence of linkage disequilibrium.

Reverse genetics: a method of investigating the genetic control of traits that begins with the nucleotide sequence of a gene and attempts to establish its function by generating and testing mutants.

SNP (single nucleotide polymorphism): a point mutation that can be targeted by molecular techniques and, thus, can be exploited as a molecular marker. SNPs are particularly amenable to high-throughput, automated profiling.

Synten: conservation of gene organization, including the linear order of genes on chromosomes (i.e. colinearity) among phylogenetically related species.

Target environment: indicates the specific environment (in terms of, for example, climatic conditions, soil characteristics, water availability and agronomic practices) where the crop is cultivated.

TILLING (targeting induced local lesions in genomes): a reverse genetics technique that enables individuals carrying a single point mutation or small insertion or deletion mutations at a target gene of known sequence to be identified within a chemically mutagenized population.

Transcriptomics: the application of techniques mainly based on microarray hybridization or cDNA sequencing that enable the level of expression of a large number of (ideally all) genes in a genome to be determined.

Water-use efficiency (WUE): index representing the number of moles of CO₂ assimilated by photosynthesis per mole of water transpired by the plant. C₄ plants and succulent plants with CAM metabolism show higher WUE than do C₃ plants.

more effectively to tailor drought-tolerant genotypes by improving the root architecture.

From a breeding standpoint, an important pitfall of most QTL studies is that the parental lines have mainly been chosen based on differences in target traits rather than on their overall agronomic value, which is often poor. Although this approach maximizes the possibility of identifying QTLs for the target traits, it does not guarantee any real progress in terms of field performance when the best-performing parental QTL allele is introgressed in the best-performing cultivars. Indeed, such alleles might already be present in the parental lines that breeders use for developing new segregating populations. Furthermore, the methods of QTL analysis currently adopted and the size of the mapping populations are for the most part inadequate for an effective and accurate detection of epistatic QTLs. Therefore, in view of these and other shortcomings of the QTL approach [16], the negligible impact of MAS on the release of drought-tolerant cultivars is not surprising.

Harnessing QTL alleles for drought tolerance from wild relatives

The success of any selection scheme relies on the availability and identification of agronomically beneficial alleles for the target traits. Traditionally, the genetic variability exploited by modern breeding to improve quantitative traits has been derived from highly selected, elite materials with a genetic basis much narrower than that of the wild relatives from which crops were originally domesticated [19]. However, beneficial QTL alleles have also been identified among wild relatives of crops [19]; therefore, the same should also hold true for drought tolerance. Such QTL alleles can be identified by means of advanced backcross QTL analysis (AB-QTL), an approach based on the evaluation of backcross families derived from a cross between an elite variety used as recurrent parent and a donor accession of a sexually compatible wild species [19]. Compared with modern cultivars, which are usually selected for high-input environments where water supply is often not a major limiting factor, wild species show morpho-physiological features for survival and adaptation to drought conditions. When considering exploiting alleles from wild species for drought-adaptive features, a careful evaluation in terms of yield once they are backcrossed in elite accessions is important.

Wild barley (*Hordeum spontaneum*), the closest relative of cultivated barley (*Hordeum vulgare*), is a valuable source of alleles for tolerance to drought [20]. A *H. vulgare* \times *H. spontaneum* backcross population was tested in rainfed conditions in three Mediterranean countries to identify wild-barley alleles able to boost yield in conditions differing widely for rainfall and evapotranspiration [21]. Among the 81 QTLs that affected agronomic traits, the wild parent contributed the beneficial alleles in 43 cases. With regards to grain yield, at six QTLs the alleles increasing yield were from *H. spontaneum*. Even in the driest environment, the *H. spontaneum* alleles at these six QTLs were not associated with earlier flowering [21], a peculiarity that in Mediterranean environments typically allows the crop to escape the negative effect of terminal drought on grain filling. These results are encouraging with regards to the possibility of

Table 1. Summary of recent QTL studies related to drought^a

Species	Cross	Environment	Main traits	Refs
<i>Arabidopsis</i>	Landsberg × Cape Verde	Greenhouse	¹² C/ ¹³ C, flowering time, stomatal conductance, transpiration efficiency	[58]
Cotton	<i>G. hirsutum</i> × <i>G. barbadense</i>	Field	¹² C/ ¹³ C, osmotic potential, canopy temperature, dry matter, seed yield	[59]
Rice	CT9993-. × IR62266-.	Field	Root morphology, plant height, grain yield	[60]
Rice	IR62266-. × IR60080-.	Greenhouse	Osmotic adjustment	[61]
Rice	CT933 × IR62266	Field	Grain yield	[62]
Rice	Zhenshan 97 × Wuyujing 2	Field	Stay-green	[63]
Rice	Bala × Azucena	Field	Grain yield, yield components	[64]
Rice	IRAT109 × Yuefu	Field and pots	Root traits	[25]
Rice	Zhenshan97B × IRAT109	Screening facility	Canopy temperature, leaf water potential, spikelet fertility	[65]
Rice	Teqing × Lemont	Field	Yield components, phenology	[66]
Maize	F2 × F252	Field	Silking date, grain yield, yield stability	[67]
Barley	Tadmor × Er/Apm	Field	¹² C/ ¹³ C, osmotic adjustment, leaf relative water content, grain yield	[68]
Wheat	SQ1 × Chinese Spring	Field	Water-use efficiency, grain yield	[69]
Wheat	Beaver × Soissons	Field	Flag leaf senescence	[70]

^aSimilar studies reported in the text have not been included in this table.

Table 2. Summary of recent genomics studies related to drought^a

Approach	Species	Main features	Refs
Transcriptome analysis	<i>Arabidopsis</i>	Expression analysis during rehydration using a microarray with ~7000 full-length cDNAs	[71]
Transcriptome analysis	<i>Arabidopsis</i>	Translational regulation was evaluated for >2000 genes by measuring the proportion of individual mRNA species in polysomal complexes in leaves of well-watered and dehydration-stressed leaves	[72]
Transcriptome analysis	Rice	Microarray-based expression analysis of ~21 000 genes in phenotypically divergent accessions for osmotic adjustment and their transgressive segregants	[73]
Transcriptome analysis	Rice	589 putative drought-stress responsive genes were localized on the physical map and their correspondence with QTLs discussed	[74]
Transcriptome analysis	Sorghum	12 982 unique cDNA clusters on a microarray were used to examine gene expression in roots and shoots in response to dehydration, high salinity or ABA	[75]
Transcriptome analysis	Sorghum	A set of unique cDNAs providing a signature of drought-responsiveness were identified among a large collection of ESTs from several libraries	[76]
Transcriptome analysis	Maize	Transcriptional profiles of endosperm and placenta-pedicel tissues in developing kernels under water stress	[77]
Proteome analysis	Sugar beet	Proteome analysis of drought-stressed leaves	[78]
Proteome analysis	Maize	Proteome analysis of the basal portion of growing leaves	[79]

^aSimilar studies reported in the text have not been included in this table.

Table 3. Web resources related to drought

Web resources	Main features
http://www.plantstress.com/	Discussions and articles on plant environmental stresses, their impact and mitigation in agriculture. Includes reference database, news, congress announcements and events.
http://www.generationcp.org/	The Generation Challenge Programme has five subprogrammes that span the spectrum of research in germplasm, genomics, bioinformatics and molecular breeding for agricultural development. The Generation Challenge Programme is focused on using genomics tools and discoveries to enhance drought tolerance in the staple crops of developing countries.
http://rarge.gsc.riken.jp/	RARGE is a website that provides services for resource data searching and presents <i>Arabidopsis</i> resource data (cDNAs, transposon mutants and microarray experiments under various stress and hormone treatment conditions).
http://rootgenomics.missouri.edu/	The website of the Plant Root Genomics Consortium is dedicated to root genetics and physiology. The aim of this consortium is to develop an understanding of the molecular mechanisms used by plant roots to acquire water and minerals from the soil and to elucidate the role roots play in adaptation to drought conditions.

adopting AB-QTL as a germplasm enhancement strategy for identifying new alleles from wild relatives that might improve yield potential under arid conditions.

Marker-assisted selection for improving drought tolerance

Once a marker-trait association has been established unequivocally, MAS reduces or eliminates the reliance on specific environmental conditions during the selection phase, a major hindrance in the conventional breeding of traits influenced by drought [5,22]. In maize, MAS has been used to introgress QTL alleles for reducing the anthesis-silking interval (ASI) (i.e. the interval between the extrusion of anthers and silks). Under drought

conditions, the ASI is negatively associated with grain yield [5]. The availability of molecular markers linked to five QTLs for ASI enabled lines with a reduced ASI to be selected [5,22]. Under severe drought conditions that reduced yield by at least 80%, the lines obtained through MAS were superior to the unselected control. However, this advantage decreased at lower stress intensity and disappeared when stress reduced yield by less than 40%. Across the water-limited trials, a few lines produced with MAS consistently outperformed the controls.

Terminal drought severely curtails rice yield, particularly under upland growing conditions. Hence, a deep root system capable of extracting additional soil moisture should positively impact yield in drought-prone areas

Box 1. Searching for drought-related QTLs

Drought-related traits are categorized as those enabling the plant to 'escape' from drought or to 'avoid' the negative effects of drought, either by maintaining a more favourable water balance or by protecting the cellular functions from dehydration [2]. Alternatively, drought-related traits can be categorized as 'adaptive' (i.e. mainly expressed under water-stressed conditions), or 'constitutive' (i.e. expressed irrespective of the water regime). Marker-assisted selection (MAS) for drought-related traits should preferably target 'major' QTLs characterized by a sizeable effect, consistent across germ-plasm and with a limited interaction with the water regime. Therefore, one of the main difficulties in identifying QTLs suitable for MAS relates to the high 'QTL \times environment' (QTL \times E) interaction that is usually reported in trials conducted under drought conditions. Once a major QTL has been identified, its accurate characterization requires its isogenization [7]. In maize, near-isogenic lines (NILs) have been produced for *root-ABA1*, a major QTL that affects root architecture, ABA concentration and grain yield across different water regimes [80,81] (P. Landi *et al.*, unpublished). Figure 1 depicts the different root architecture of the NILs. A more systematic and rapid approach that can be used to generate NILs for different traits simultaneously is through the construction of an introgression library (IL) (i.e. a number from ~80 to 150, according to the genome size of the target species) of highly isogenic lines obtained with five or more backcrosses, with each line carrying a small portion (~20–30 cM) of a donor genome in an otherwise common genetic background [82]. In maize, QTLs for root architecture have been identified through the evaluation of a recently assembled maize IL (S. Salvi *et al.*, unpublished).



Figure 1. Effect of the isogenization of *root-ABA1* on the root architecture of Os420 and IAB078, the two parental lines that were crossed to obtain the mapping population that allowed for the identification of this major QTL. The (+/+) and (-/-) indicate the *root-ABA1* alleles increasing and decreasing root mass, respectively. Scale bar = 5 cm. Figure reproduced, with permission, from Ref. [81].

where residual moisture is available in deeper soil layers [2]. Several studies have investigated QTLs for root traits in rice and their associated effects on other drought-related traits and, in some cases, also grain yield [23–25]. However, only a handful of these studies have used MAS to introgress the desirable QTL alleles into different genetic backgrounds and validate their effects. MAS was used to transfer four QTL alleles for deeper roots from 'Azucena' [26,27], a japonica upland cultivar that is well adapted to rainfed conditions, into 'IR64', a rice cultivar characterized by a shallow root system. Phenotypic evaluation of root traits indicated that MAS was successful for three QTL regions. When grown under aerobic or drought-stressed field conditions, the MAS-generated lines that were characterized by a root mass greater than that of 'IR64' below 30 cm outyielded 'IR64' and MAS-generated lines with the same root distribution as 'IR64' [27]. A marker-assisted backcross (MAB) programme was implemented to improve the root morphological traits and, hence, the drought tolerance of 'Kalinga III', an upland rice variety. In contrast to the previous study, the recurrent parent in the MAB had not been previously used for QTL mapping. Also in this case, 'Azucena', the donor parent, provided four QTL alleles for improved root length. Of the four 'Azucena' introgressed segments, only one on chromosome 9 significantly increased root length under both irrigated and drought stress treatments, confirming that the 'Azucena' QTL allele for root length is effective in a novel genetic background. The remaining introgressed QTL segments did not show significant effects on root length, possibly because of similar QTL alleles in the parents and/or epistatic interactions with QTL alleles from 'Kalinga III' [28]. These results clearly indicate that the effect of QTL alleles can be influenced by the genetic background of the accessions used in the breeding programme. The relevance of epistatic interactions in determining the effects of a target QTL originally detected in a mapping population can be tested by association mapping (Box 2). In this case, a core collection of accessions representative of the cultivated varieties are profiled with molecular markers to determine their genotype at target QTLs and a genotype–phenotype association is statistically verified. In such a context, the QTL allele or alleles showing consistent genetic effect should be reliably identified. Recently, 'Mapping As You Go' (MAYG), a new approach for MAS of complex traits, has been also proposed for overcoming the problems associated with epistatic QTLs [29]. The MAYG approach partially accounts for the presence of epistasis and the $G \times E$ interaction by implementing MAS so that the estimated values of QTL alleles can evolve as the current germplasm evolves over cycles of selection. The effectiveness of the MAYG method for a range of genetic models has been estimated through simulation [29].

Understanding the functional basis of drought tolerance through genomics

Besides being exploited in MAS projects, major drought-related QTLs can be considered for cloning (Box 2) with the aim of manipulating the target trait more directly by genetic engineering. The identification of candidate genes for the QTLs and the elucidation of their functional role can be

Box 2. Cloning QTLs for drought-related traits: why and how?

Cloning a drought-related QTL substantially contributes toward a better understanding of the genetic and functional basis of the response of a plant to drought. Furthermore, the sequence responsible for the QTL becomes available for genetic engineering and/or mining for the most desirable alleles within germplasm collections.

Until now, two approaches have been mainly used for the molecular dissection of a QTL: positional cloning and association mapping (Figure 1). Positional cloning begins with the production of a large population in a near-isogenic background where only the target QTL segregates. A large number of progeny (>1000) with recombination events and molecular markers in the target region enable us to identify the genetic and physical interval co-segregating with the QTL. The availability of the genome sequence facilitates the connection between the genetic and physical information. When the genome sequence is not available, genomic libraries (e.g. BAC clones) are used. Candidate genes or sequences that co-segregate with the QTL are then functionally tested with reverse genetics tools (e.g. identification of knockouts, RNAi and TILLING) and/or ectopic expression.

Association mapping based on linkage disequilibrium (LD) seeks to establish a statistical association between allelic (or haplotype) variation at a locus and the phenotypic value of a trait across a large enough sample of unrelated accessions [83]. The LD approach offers two distinct advantages: the survey of multiple alleles in a single analysis and avoidance of the time-consuming preparation of mapping populations. In the presence of high LD (~100 kb or more), association mapping can only provide coarse mapping information; however, when LD is low (~10 kb or less) the resolution power is sufficiently high to assign the QTL to an interval containing one or a few genes. Analysis of candidate genes has already provided interesting results [83,84]. Association mapping should greatly benefit from the introduction of high-throughput platforms that are able to profile a multiplex of single nucleotide polymorphic (SNP) markers. Techniques such as Eco-TILLING [46] are also available to streamline the identification and scoring of new alleles at target genes or sequences.

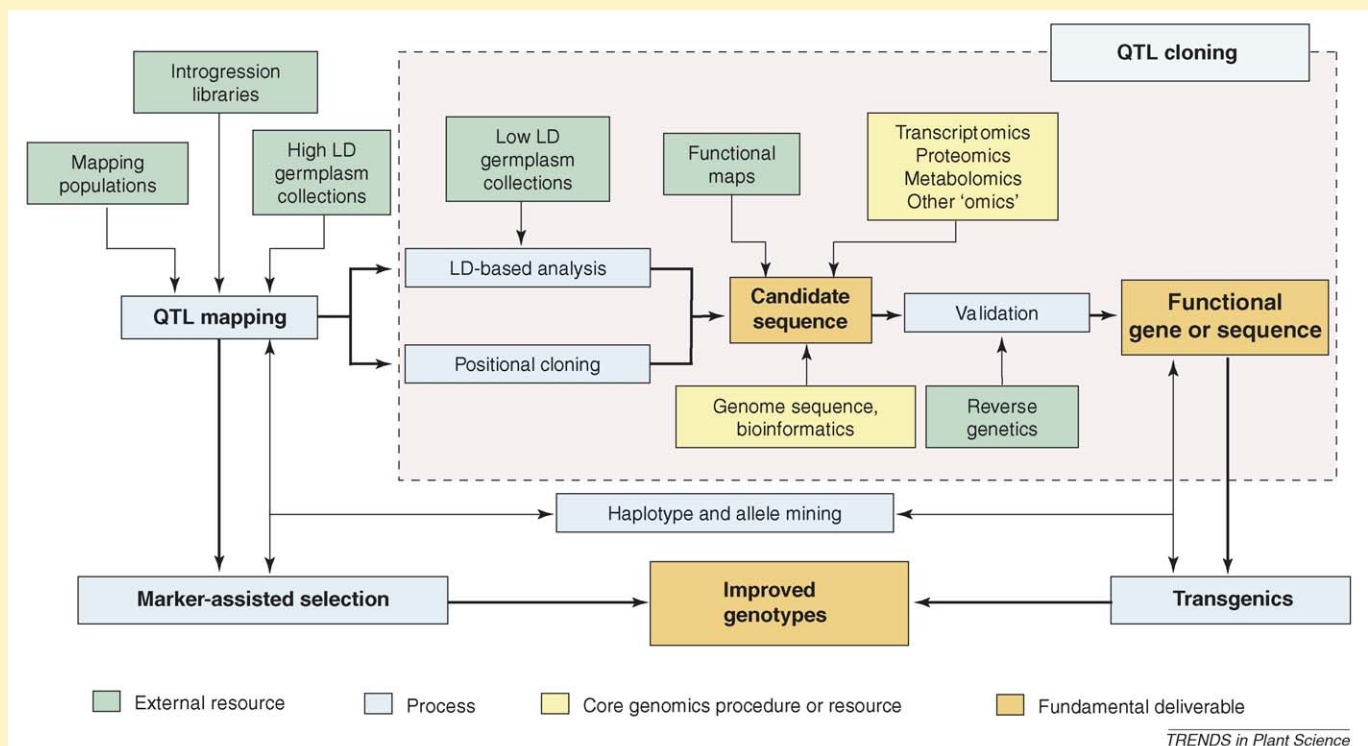


Figure 1. A schematic representation of the position and role of QTL cloning in the current framework of marker-assisted breeding activities aimed at crop improvement for quantitative traits such as drought tolerance.

facilitated by combining QTL maps with the so-called functional maps (i.e. maps enriched with genes potentially involved in controlling the target trait or with fully annotated genomic sequences) [9,11,30]. For example, a comparative analysis of QTL data for root traits of three rice-mapping populations with one parent ('Azucena') in common, combined with the screening of ESTs and cDNA clones has identified two genes for cell expansion (*OsEXP2* and *EGase*) that were mapped within the support intervals of QTLs for root traits common to the three populations [31].

Progress in the mass-scale profiling of the transcriptome, proteome and metabolome has allowed a more holistic approach in investigations of drought tolerance based

on the measurement of the concerted expression of thousands of genes and their products. High-throughput mRNA profiling has been applied to investigate the changes in gene expression in response to dehydration [32] (Table 2). An example on how transcriptome analysis can advance our understanding of the physiology underpinning drought-related traits has been recently provided by the expression profiling of primary root apices in maize [33]. Collectively, the transcriptome profiling experiments conducted on drought-stressed plants have highlighted the central role of transcription factors (TFs) while unveiling the complex hierarchy of the regulatory network that differentially modulates the expression of dehydration signature genes in a tissue-specific manner [34]. In this

respect, laser-capture microdissection is a major technical breakthrough: the technique allows the profiling of specific cell types [35] – a feature particularly important when investigating genes encoding for TFs expressed weakly and/or in a cell-type-specific fashion. Along the same line, an interesting application of transcriptome analysis is the identification of the so-called eQTLs (expression QTLs, i.e. QTLs that influence the level of gene expression) [36]. However, the cost of profiling the large number of samples required to identify eQTLs is still too high for routine application of this approach.

Deciphering gene function can also be facilitated by information gathered through profiling the proteome and metabolome. In rice, >2000 proteins were detected in leaf tissue [37]. The most marked, drought-induced changes were identified for the actin depolymerizing factor (ADF), the chloroplastic glutathione-dependent dehydroascorbate reductase and a homologue of the S-like ribonucleases. The level of ADF was also influenced by drought in another study conducted on rice [38]. In this case, ADF concentration was higher in a drought-tolerant rice cultivar before stress application and increased further in leaf blades, leaf sheaths and roots after exposure to drought, suggesting that ADF is one of the target proteins induced by drought stress. Profiling the proteome of a mapping population offers the opportunity to identify QTLs influencing protein quantity (PQLs, Protein Quantity Loci). In water-stressed maize, the *Asr1* gene, a putative transcription factor, has been shown to co-localize with a PQL for the ASR1 protein and a QTL for ASI and leaf senescence [39].

With regards to metabolomics, the present technology enables the profiling of ~2000 metabolites in a single sample [40]. Although to date no drought-specific study has been carried out on such a large scale, several studies have investigated the role of specific metabolites (e.g. sugars) and the corresponding enzymes during the reproductive phase of cereal crops [41,42]. The susceptibility of early developing grain to water stress is a major problem in maize where a shortage in assimilate supply has been indicated as the likely cause for insufficient grain filling and/or sterility [41]. In this respect, invertase activity in the developing kernel has been shown to be an important limiting factor for grain yield in maize exposed to drought [43]. Among the QTLs for invertase activity described in maize, one mapped near *Ivr2*, an invertase-encoding gene [44,45]. Furthermore, colocalization between the activities of two enzymes (sucrose-P synthase and ADP-glucose pyrophosphorylase) involved in carbohydrate metabolism and corresponding structural genes has been reported in young maize plants subjected to water deficit [45].

Overall, the results of the studies discussed here provide compelling evidence of how genomics-based approaches can contribute novel information to identify candidate genes and elucidate their functions and regulation under water-limited conditions. Further information on the role of candidate genes can be obtained through EcoTILLING, a platform for classifying SNP (single nucleotide polymorphism) haplotypes at target loci and ascertaining their effect on the phenotype [46].

Future challenges and perspectives

Despite the spectacular progress of genomics during the past decade, the implementation of accurate, high-throughput phenotyping for drought tolerance traits remains a major challenge for QTL mapping studies. This factor should not be underestimated for a more effective exploitation of the potential benefits deriving from QTL discovery and/or MAS. Among the phenotypic traits that could benefit most from the application of MAS, root architecture and photosynthetic efficiency under drought are interesting targets in view of their important role in determining the biomass of crops and the difficulties in improving these traits with conventional approaches [2,4]. Additional traits whose enhancement would be facilitated by MAS include osmotic adjustment, relocation of stem reserves and leaf senescence. QTLs for these traits have been identified [47].

From a mapping point of view, we expect that increasing attention will be devoted to the construction of consensus maps integrating the QTL information provided by different populations [16,22]. In maize, a consensus map has been developed to identify the chromosome regions that have been shown most frequently to affect drought tolerance [48]. Comparative mapping among different species poses several challenges and opportunities. Although colinearity between *Arabidopsis* and both rice [49] and maize [50] has been severely eroded, comparative mapping between rice and the other grasses [51] should facilitate the identification of candidate genes and QTL cloning in cereals. However, chromosomal rearrangements could in some cases undermine the use of rice as a model for cross-species transfer of information in evolutionarily non-conserved regions [52]. For dicot crops, opportunities are available for species closely related to the model plant *Arabidopsis*, particularly for genes and QTLs governing the perception of dehydration and/or the activation of the signal-transduction cascade that triggers the adaptive response to drought. A recent study in *Arabidopsis* reported the cloning of a QTL able to regulate transpiration efficiency while maintaining biomass production, hence uncoupling the usually negative association between transpiration efficiency and both stomatal conductance and biomass production [53].

The future contribution of genomics to the release of drought-tolerant cultivars will depend on the capacity to identify agronomically valuable QTL alleles and their use in a MAS-based 'breeding by design' approach [54]. To some extent, this could be regarded as an evolution of the so-called 'ideotype' breeding [55], the main difference being that we now can, based on molecular profiles, consciously select and move specific alleles from one variety to another to pyramid the best alleles in agronomically superior genotypes, in a sort of molecular jigsaw puzzle. However, in the case of drought tolerance, this concept of 'breeding by design' is still a long way from being routinely applicable given our rudimentary understanding of the physiology of yield in water-limited conditions and the difficulty in predicting the phenotypic value of a new assembly of alleles. The application of robust QTL-based crop model that can predict gene-to-phenotype relationships and that can narrow the genotype-phenotype gap can partially overcome difficulties in predicting the

phenotypic value of a new assembly of alleles [18,56,57]. It is thought that integrating crop modelling with genomics-based approaches will enhance the effectiveness of MAS and breeding efficiency while improving our understanding of phenotypic plasticity and its role in adapting crops to a wide range of water regimes.

The past decade has witnessed an increase in QTL studies for drought-related traits and the first encouraging results in QTL cloning [7]. With its far-reaching implications, QTL cloning enables us to better understand and more effectively manipulate the traits influencing drought tolerance. We are confident that molecular-assisted breeding will help us to face the challenges posed by the decreasing availability and escalating price of irrigation water more effectively as well as the increasing demand for food, fibre and biomass. On a more realistic note, the successful exploitation of genomics to enhance drought tolerance will only be possible within a coherent, interdisciplinary context able to provide a thorough understanding of the factors limiting crop yield in drought-prone environments.

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