Genetic approaches to crop improvement: responding to environmental and population changes

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Abstract | Crop production is threatened by global climate change, and recent demands for crops to produce bio-fuels have started to affect the worldwide supply of some of the most important foods. How can we support a growing human population in such circumstances? One potential solution is the improvement of crops to increase yield from both irrigated and non-irrigated lands, and to create novel varieties that are more tolerant to environmental stresses. Recent progress has been made in the isolation and functional analyses of genes controlling yield and tolerance to abiotic stresses. In addition, promising new methods are being developed for identifying additional genes and variants of interest and putting these to practical use in crop improvement.

Quantitative trait locus (QTL). A genetic locus controlling a complex trait (such as plant height or grain yield), which is typically affected by more than one gene and also by the environment.

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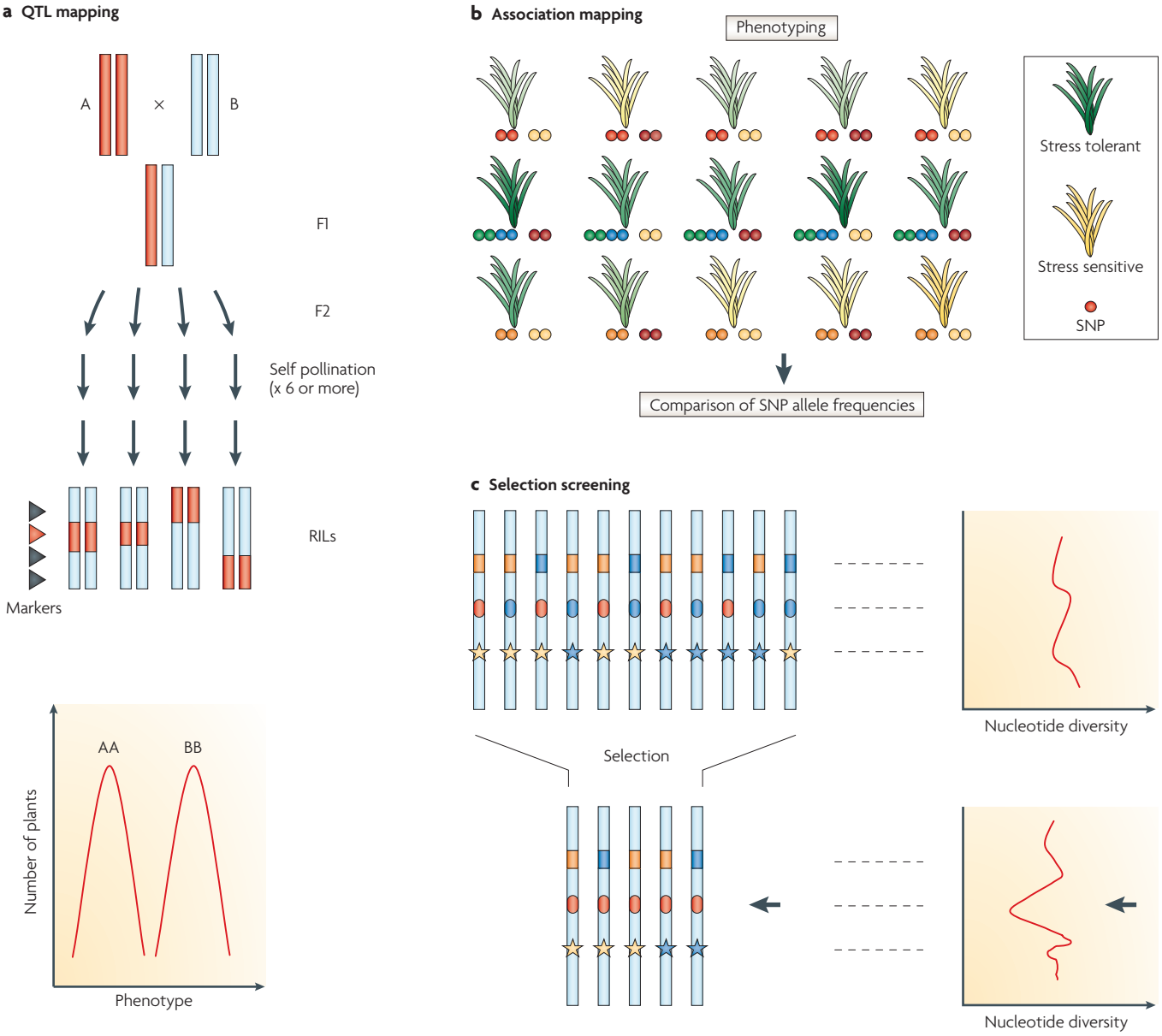
In addition to an increasing world population, there are numerous reasons for serious concern about sufficient future global production of food from crop plants[1–4]. First, the availability of arable land is decreasing because of non-sustainable farming, soil erosion and degradation[2]. Second, the availability of water for agriculture will decline[1]. Third, global climate changes will not only seriously affect crop growth but will also threaten the conservation of cultivated land[5,6]. Droughts, storms, floods, heat waves and rises in sea-level are predicted to occur more frequently, and salinity and other soil toxicities are likely to be much more problematic in some areas. In semi-arid regions, reductions in yields of primary crops, including maize, wheat and rice, are predicted in the next two decades[7]. Lastly, demands for bio-fuels as substitutive energy sources will be increased, reducing the land available for cultivating food crops[4].

To overcome these problems, new agricultural technologies will be needed to ensure global food security, in addition to efforts to conserve water and lands. In particular, crop improvements that confer tolerance to environmental stresses and soil toxicity, as well as high yield and biomass, will be required. To achieve this goal, the loci that influence these traits need to be identified to provide an understanding of their molecular mechanisms. Moreover, germplasm sources carrying trait-enhancing alleles must be identified, and their behaviour in appropriate genetic backgrounds and environments documented. Here, we first discuss established and emerging approaches that can be used to identify the genetic components controlling advantageous traits and the possibility of using these components for crop improvement. We then review recent progress on the identification of genes or loci involved in important quantitative traits of two main categories. The first of these covers recently identified genes that control yield. The second main area that we focus on is the genetic architecture of traits involved in tolerance to abiotic stresses, especially to water stress and soil stress. These stresses include submergence, drought, salinity and other soil toxicities, such as boron and aluminium toxicity, all of which are expected to be more problematic in the future. We overview how these studies have contributed to our understanding of the underlying biology, and address the possible application of such knowledge to crop improvement, including breeding strategies and genetic modification. The aim of this Review is to provide a critical overview of the approaches available and outline the approaches that promise to increase future chances of success.

Identifying useful genetic components

Established approaches — molecular genetic approaches and QTL mapping. The identification of genes that are responsible for important agricultural traits has been mostly conducted by traditional molecular genetics (forward and reverse genetic screens) for discrete traits and by quantitative trait locus (QTL) mapping for complex traits[8–10] (BOX 1). In general, the traditional methods are powerful enough to reveal the genes that are involved

Box 1 | **Methods for identifying genes involved in specific traits**



QTL mapping

QTL mapping relies on statistical linkage analyses among quantitative traits of interest and genetic markers, using a population that carries genetic mosaics derived from parental varieties, such as second generation (F2) plants or recombinant inbred lines (RILs). In part **a** of the figure, parental varieties (A and B) are used for the preparation of RILs for mapping. Segments derived from parental chromosome are represented by two colours (light blue and red). At each genetic locus (indicated by arrowheads), phenotypic variance for plants with the genotypes AA or BB, is scored. At a QTL, indicated by the red arrowhead, graphs representing phenotypic variations for AA and BB are separated, as shown in the bottom section of part **a**. It should be noted that RILs, rather than the F2 or F3 population, are needed to evaluate genotype-by-environment interactions.

Association mapping

Association mapping, also called linkage disequilibrium (LD) mapping, relies on correlation between a genetic marker and a phenotype among collections of diverse germplasm. In part **b** of the figure, the principle of association mapping is illustrated. Phenotypes are scored for plants that have also been genotyped (SNP genotypes are represented by coloured circles). For simplification, the plant variants shown here are homozygous at each locus. Association scanning is performed by comparing phenotypic scores respective to each haplotype. Data sets are analysed using statistical methods, which have been designed to deal with the population structure.

Selection screening

Selection screening identifies genes that show signatures of selection. This approach is based on the theory that target loci of selection show decreased nucleotide diversity and increased LD after strong selection, such as during domestication and subsequent crop improvement. Whereas association mapping is a trait-oriented method, selection screening is primarily dependent on DNA sequence polymorphism. In part **c** of the figure, loci for monitoring polymorphisms are represented by circles, rectangles, and stars with different colours over a genomic region. A selected gene locus is expected to undergo loss of diversity after selection (arrow), whereas other loci are supposed to be neutral and retain high nucleotide-sequence diversity in the population.

in a particular function, whereas QTL mapping reveals the effects of genetic variants on complex traits, which include most agronomic traits.

Molecular genetic approaches are relatively straightforward in model plants such as *Arabidopsis thaliana* and rice, in which genomic sequence information is available and transformation techniques are well established[11,12]. Maize is also useful for genetic studies as plentiful resources and tools have been developed, including large-scale collections of mutants (based on chemical mutagenesis and transposon insertions) for both forward and reverse genetic screens[13], and sequencing of the entire genome has just been completed (see below). In *A. thaliana* and rice, insertion lines generated

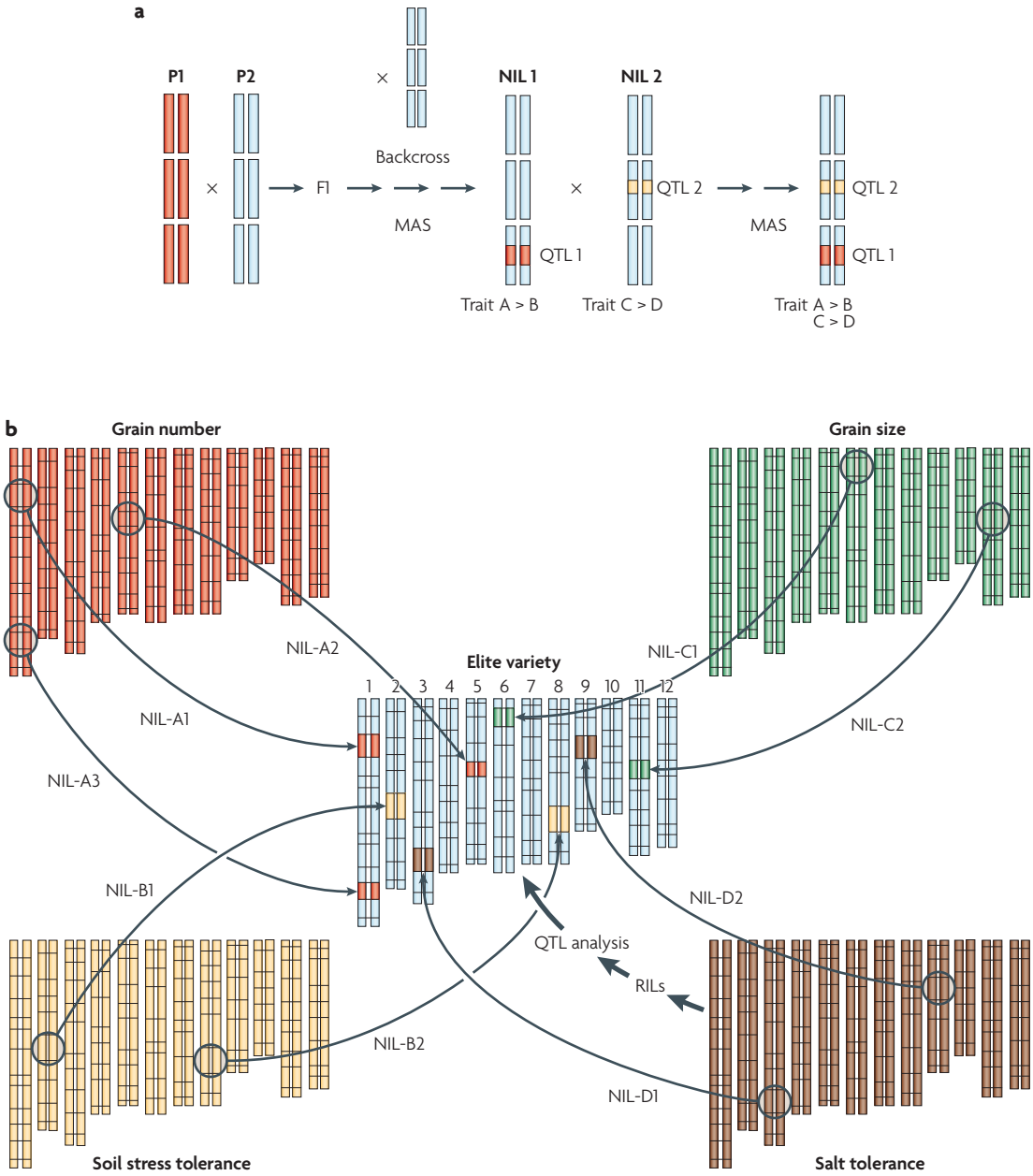


Figure 1 | **QTL pyramiding. a** | A nearly isogenic line (NIL), carrying a QTL responsible for an advantageous trait (QTL 1) from parental line 1 (P1) is obtained by marker assisted selection (MAS) after backcrossing — using parental line 2 (P2) as a recurrent parent — of the first generation (F1) progeny of the parental lines. The resulting NIL (NIL 1) has a chromosome segment that includes QTL 1 but is otherwise identical to the P2 genetic background. QTL pyramiding is achieved by hybridization to another NIL (NIL 2) carrying a different advantageous QTL (QTL 2) and by subsequent MAS, generating a line with a combination of beneficial traits. **b** | Ultimate QTL pyramiding, which involves the introduction of multiple advantageous traits from a range of different varieties into an elite variety for which improvement is desirable. The map positions of loci involved in advantageous traits must be determined; this can be achieved by QTL analysis using different populations of recombinant inbred lines (RILs) or second generation (F2) variants, each of which can be derived from an elite variety crossed to another variety with the trait of interest.

by the use of T-DNA (the modified, transferable DNA of some species of bacteria) are available, in addition to mutant libraries prepared by chemical mutagenesis and transposon insertions. Moreover, systematic transgenic approaches using enormous cDNA collections have been enabled recently in *A. thaliana* and rice. For example, the full-length cDNA overexpressor (FOX)-hunting system[14,15] involves screening a collection of transgenic lines in which full-length cDNAs are overexpressed at random. However, transgenic approaches for both forward genetic screens and reverse genetic studies are not yet practical in many crop species (such as wheat, barley and sorghum), in which gene manipulation technologies are inefficient or not available. Another limitation is reliable phenotyping, which is still a laborious, time-consuming task; innovations in this area will be important for future progress.

Identifying existing genetic variation by QTL mapping (BOX 1; FIG. 1) is important because, unlike molecular genetic approaches, this can provide a basis for crop improvement through conventional breeding approaches[8–10]. The feasibility of QTL mapping depends on the availability of genetic markers and populations of segregating individuals showing measurable phenotypic variation, whereas positional cloning of QTL genes is facilitated by sequence information and/or a physical map of the genome. By these criteria, rice is the leading species among cereal crops, because its complete genome sequence is available[16]. As the genome structure is conserved between rice and other cereals, such as maize, barley, wheat and sorghum[17], sequence information from the rice genome is also useful for comparative mapping in these monocotyledonous (monocot) crops. Owing to recent advances in sequencing technology and ongoing genome sequencing projects, the availability of fundamental information for mapping and cloning is being increased for a wider range of crop plants[18,19]. Recently, draft sequencing of the genome of sorghum and maize has been completed (see the Phytozome, US department of Energy (DOE) Joint Genome Institute (JGI), Maizesequence, and Maizegenome web sites for details). However, gene identification by QTL mapping in crop species is still challenging, especially for loci controlling abiotic stress tolerance. This is partly due to difficulties in controlling growth conditions for many crops in the field. For example, because plant responses to salt and drought stress overlap with responses to low- and high-temperature stress[20,21], changes in humidity and temperature might easily affect these stress responses, causing noisy backgrounds for phenotypic evaluation and results that are not reproducible. Thus, repeated evaluation of each trait using inbred lines, which is more laborious and time consuming, is required to obtain convincing results.

Emerging approaches — association mapping and selection screens. In addition to these well established mapping methods, two additional approaches that are based on population genetics and that make use of genetic diversity have recently emerged: association mapping and selection screens (BOX 1).

In brief, association mapping relies on correlation between a genetic marker and a phenotype of interest among plants that are derived from collections of diverse germplasm[9,22]. Thus, large numbers of alleles can be tested — this is in contrast to QTL mapping, in which only parental alleles are tested. Association mapping theoretically also allows easier fine mapping than the classical biparental-cross approaches, in which a large number of segregants are often needed. This is because hundreds or thousands of generations are likely to have passed since the establishment of association between a marker and a linked causative allele, allowing time for large numbers of recombination events during meiosis. Such events break down association between a causal variant and a genetic maker that is not tightly linked to it. In association mapping, the marker density and experimental design are determined by linkage disequilibrium (LD) patterns[23,24], which reflect recombination history and demographic factors, including population history and inbreeding. Using large marker sets, association mapping can potentially be performed across the whole genome, an approach that is currently being widely put to use in human genetics[25] (BOX 1).

The feasibility of genome-wide association mapping in *A. thaliana* was recently tested using 95 accessions[26,27] in a search for associations with flowering time and pathogen resistance, and known genes with major effects on these traits (*FRI*, *RPM1*, *RPS2* and *RPS5*) were successfully identified. Thus, whole-genome association mapping in *A. thaliana* is powerful enough to generate a list of candidate QTLs, although population structure in this species is an important challenge in terms of eliminating false positive results[27,28]. Among cereal crops, maize is most suitable for association analysis because it is an outcrossing species exhibiting a high recombination rate and rapid decay of LD (<1 kb)[22]. In both maize and other crop species — including rice, barley, sorghum and soybean, which are self-crossing — diverse germplasm or genotype panels are also being established for whole-genome association mapping[29]. In self-crossing crops, however, the scale of LD is relatively large, leading to lower mapping resolution. In addition, in all crop species, population structure might be strongly affected by artificial selection for cultivation and by geographical factors, which are likely to result in spurious associations being detected. The risk of such false associations can be decreased by the aid of appropriate statistical methods. For example, in the studies of *Dwarf8* polymorphisms that are associated with variation in flowering time in maize, the number of false positive associations was decreased by controlling for population structure[30]. More recently, a mixed-model method for controlling error rates has also been developed[28].

It should also be noted that this method of mapping identifies an association between a trait and a genetic marker, but not a causal relationship between the two. Therefore, it is desirable to verify the function of the associated genes by linkage analysis (as exemplified with the maize *PSY1* gene for phytoene synthase that is associated with endosperm colour[31,32]), or by transgenic studies. In these contexts, association mapping is

likely to soon become more powerful in maize (see the Panzea web site) and in rice (see the RiceHapMap web site). Supporting this, haplotypes at the *lycopene epsilon cyclase* (*lycE*) locus have recently been revealed to be responsible for variations in the content of vitamin A precursors in maize grains, by an association study of the candidate genes combined with QTL analysis and chemical mutagenesis[33].

Another recently developed method identifies genes of interest by looking for signatures of selection[34–36]. This type of screen is based on the theory that loci that have been targets of selection show a decrease in nucleotide diversity and increased LD after strong selection, such as during domestication and subsequent crop improvement[37] (BOX 1). Domestication genes can be identified by comparing nucleotide sequence diversity between a crop species and extant populations of wild relatives as a proxy of the ancestor species. For example, in a large-scale selection screen, eight genes with various functions were identified as candidates for genes that are subject to selection during domestication in maize[35]. However, to use the knowledge gained from these screens for crop improvement, the traits that are affected by the selected gene need to be established, along with an understanding of the underlying biology and subsequent linkage analysis. Moreover, this method might not be suitable for all crop species. For example, in a selection screen in sorghum, no compelling evidence for selection was found for 371 loci that had been examined[38]. In addition, it has been suggested that non-neutral patterns of diversity can be caused by demographic factors as well as by selection. Thus, the usefulness of this approach might be limited to specific species or populations for which the demographic history has been studied and can be taken into account, such as maize and *A. thaliana*. Finally, this method relies on selection having taken place far enough in the past for sufficient genetic changes to have accumulated.

Transferring useful traits for crop improvement

To transfer genetic information conferring advantageous traits to a cultivar of preference, both transgenic (genetic modification) and non-transgenic approaches can be used. The non-transgenic approach is based on hybridization of two varieties carrying advantageous QTLs or useful gene alleles, and subsequent marker-assisted selection of those genetic components (FIG. 1). The ultimate selection of highly advantageous combinations of QTLs is called QTL pyramiding[10]. This approach does not necessarily require the identification of the causal genes but can be carried out using small chromosome segments, each of which carries only one QTL. Theoretically, superior alleles from several or more different parental varieties can be introduced into an elite variety of interest (FIG. 1b). Disadvantages are that this method is labour intensive and time consuming, and that the transfer of relevant alleles by hybridization is restricted to the same species. More importantly, the effectiveness of this approach depends on the genetic architecture of the trait. This is especially important because, if the total variation for a trait was based on many genes of small effect, this strategy would not be straightforward, mostly because of complexities that arise owing to genetic and/or genotype-by-environment interactions. In such a case, QTLs can be mapped, but it does not mean that these loci are ready to be used for breeding. The problem is that it is often hard to track these loci in substitution lines or in nearly isogenic lines (NILs), probably because the relevant genetic interactions are lost. Only when the effects of QTLs are significant enough in NILs are they really available for breeding and also for gene cloning. The same is true when a QTL is transferred into a variety with a different genetic background.

By contrast, the transgenic approach does not require hybridization, but does require identification of the gene responsible for an advantageous trait. Even genes from non-plant species can potentially be used. In principle, combinations of several beneficial genes can be transferred into the same plant. However, not all crop species or cultivars are easily transformed. In cereal crops other than rice, transformation methods have either not been established (for example, in sorghum) or existing methods have only low efficiency (for example, in wheat and barley). Even in rice, some cultivars are transformed only at low levels. Thus, technical advances in gene manipulation will be required for improvement of those plants in the future. Another potential limitation is that the function of homologous genes is often not exactly the same among plant species (or, indeed, among plant and non-plant species), which might cause unexpected and unwanted side-effects. For practical application, marker-assisted backcrossing of a transgene into a commercially viable variety is often needed. There is also the issue of the public acceptance of genetically modified crops, which is beyond the scope of this Review.

Yield improvement

Among the traits affecting crop yields, we focus on those that are linked to genetic programmes controlling the size and number of reproductive organs rather than traits that are indirectly involved in yield stability, such as the semi-dwarf trait. However, it should be noted that moderate reduction of plant height in cereal crops is important to avoid lodging and thereby to increase yields, as represented by the 'Green Revolution' trait. Genes involved in this form of yield improvement in wheat or rice are known to encode a factor that interferes with the signal transduction pathway of the growth hormone gibberellin (GA) or with production of GA, as reviewed previously[39]. In terms of developmental aspects, terminal-branching pattern and fruit-size control seem to be the predominant determinants for the yield improvement of fruits and grains. In rice, for example, two basic traits largely affect grain yield: the number of grains per panicle and the size of the grains. The number of grains mostly depends on the branching pattern of panicles, which is determined by the activity and size of the shoot meristem as well as the timing of transition from shoot to flower[40–42], whereas the size of the grains reflects cell-division activity. Studies on the genetic control of inflorescence structure in maize and other grasses have recently been reviewed[43,44].

Nearly isogenic line
(NIL). A line carrying an isolated homozygous segment that contains a target QTL of a parental chromosome; other than this QTL, the line has the other parental genetic background.

A pioneering study for crop yield was the identification of a gene controlling fruit size in tomato by QTL mapping[45]. This gene — *fw2.2,* the first QTL gene to be positionally cloned in plants — is responsible for 30% of the difference in fruit mass between wild and cultured tomato. *fw2.2* encodes a protein with partial homology to the human RAS oncoprotein, and acts as a negative regulator of cell division[45]. Subsequent studies showed that the accumulation of *fw2.2* transcripts varied in terms of timing and quantity between the two accessions[46], and that expression levels are negatively correlated to fruit mass in an artificial gene dosage series[47].

In cereal crops, many QTLs affecting yield and their map positions have been listed in the Gramene database. Recently, rice genes controlling grain mass have been identified by QTL mapping. *gs3* was identified as a major QTL for grain length and a minor QTL for grain width and thickness[48]. *gs3* encodes a putative transmembrane protein, which might function as a negative regulator of the growth of grains. Another QTL controlling grain width and weight, *gw2*, has also been identified[49,50]. *gw2* encodes a RING-type ubiquitin E3 ligase, suggesting that it functions through degradation of an unknown target protein by the 26S proteasome. The encoded protein is suggested to be a negative regulator of cell division in spikelet hulls, as loss of function increases cell division and thereby grain mass.

In terms of branch numbers, *Gn1a* has been identified as a major QTL affecting the number of secondary and tertiary branches in rice, and thereby grain numbers per panicle[51]. *Gn1a* encodes cytokinin oxidase/dehydrogenase (OsCKX2), an enzyme that degrades cytokinin, the phytohormone that promotes cell division. A rice variety with high grain yield, Habataki, has a partial loss-of-function allele of *ckx2*, the gene encoding OsCKX2, which is expressed at lower levels than in Koshihikari, a variety with a lower grain yield (BOX 2). The reduced expression causes cytokinin accumulation in inflorescence meristems and increases the number of reproductive organs, accounting for the increase in yield. Moreover, a Chinese variety, 5150, which has more grains per panicle than Habataki, was shown to have a null allele of *ckx2*. The low level expression of *cks2* in 5150 is also correlated with high levels of cytokinin in the inflorescence meristem. Ashikari *et al.* achieved successful QTL pyramiding for yield improvement by introducing the rice Green Revolution gene, *sd1*, which reduces plant height in the Koshikari genetic background, thereby generating a variety carrying two beneficial traits: high yield and lodging resistance. This new variety is now being evaluated in further field trials for food production in Japan.

QTL-mapping studies for grain size and for secondary and tertiary branching have revealed that control of the cell-division cycle is a common key regulatory mechanism that influences two aspects of crop yield. However, it is still unknown how the factors that have been identified regulate cell division in each developmental stage. It also remains to be elucidated whether the same regulatory mechanism operates in other crop species, and whether the knowledge gained can therefore be put to use in yield improvement across different species. Recent works have shown that the number of primary branches in panicles is probably under the control of a few major QTLs (M.M., unpublished observations), and it will be interesting to see whether these QTLs are also involved in the cell cycle. For further yield improvement in rice, the introduction of a combination of the major QTLs for the different but related traits — such as *gs3*, *Gn1a*, and QTLs for primary branching or additional traits affecting yield that are likely to be identified soon[52] — will be a promising approach. Further improvement should also be possible, if superior gene alleles could be found and introduced using an allele-mining approach (BOX 2).

Improving stress tolerance

Drought tolerance. Drought, together with soil salinity, is one of the stresses that threaten worldwide crop productivity most severely. In terms of physiology, both drought and salt stresses, as well as heat or cold stress, produce overlapping responses in plants, including accumulation of osmoprotectants and heat shock proteins, some of which are mediated by the actions of the phytohormone abscisic acid (ABA)[20,21,53–56]. Extensive studies into the signal transduction and transcriptional regulation involved in these responses, mainly in *A. thaliana*, have been reviewed recently[21,55,56], and thus are not described here. Enhancement of these responses by gene manipulation often results in improved tolerance to abiotic stress, but the significance of many of the genes identified by these studies has not yet been verified in crop plants in terms of availability for breeding. An exception is NFYB2, a transcription factor of maize, which confers drought tolerance, leading to yield improvement under water-limited conditions in field efficacy trials[57]. This factor was identified as being orthologous to the *A. thaliana* transcription factor NFYB1, which was originally isolated by a systematic transgenic approach in a screen of more than 1,500 transcription factors that were expressed constitutively in *A. thaliana*.

Among crop species, sorghum has been studied as a model for drought resistance because of its adaptation to hot and dry environments. A particularly relevant trait conferring drought tolerance towards improvement of sorghum is the 'stay green' trait. This is characterized by delayed leaf senescence during grain ripening under water-limited conditions, which ensures better grain filling and is often associated with resistance to charcoal rot and lodging[58]. Physiologically, this trait has been suggested to correlate with sugar content of stems and with levels of cytokinins[58]. Consistent with this, drought tolerance is enhanced by delaying leaf senescence when an isopentenyltransferase gene for cytokinin synthesis is expressed under the control of a stress- and maturation-induced promoter[59]. Several QTL-mapping studies for stay green identified four major QTLs, designated *Stg1*, *Stg2*, *Stg3*, and *Stg4*, which account for approximately 20%, 30%, 16% and 10% of the phenotypic variance, respectively[60]. Although the genes responsible for these major QTLs have not yet been identified, it is expected that recent progress in the sorghum genome sequence

project (see the Phytozome web site) will facilitate fine mapping of QTLs and subsequent gene identification.

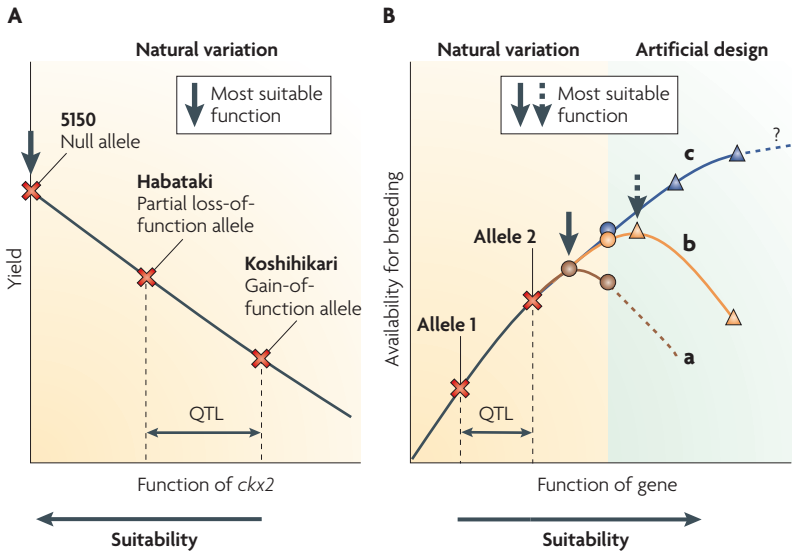
In maize, a major QTL designated *root-ABA1* is involved in root architecture, ABA concentration and other traits according to water availability[61]. This QTL accounts for 32% of the phenotypic variation in ABA concentration in the leaf. Other QTL studies in several crops for drought tolerance or drought-related traits have been summarized in recent reviews[58,62]. More recently, major QTLs with large effects (accounting for 32–33% of the genetic variation) for grain yield under drought stress conditions in upland and lowland rice have been reported[63,64]. However, the genes responsible have not yet been identified in any of these studies. Whether the genes underlying these QTLs prove to be novel or identical to previously identified genes, they are promising opportunities for improving drought tolerance in various crops.

Submergence tolerance. Submergence stress owing to flash flooding is a major constraint to rice production in south and southeast Asia. It causes a reduced oxygen

Box 2 | **Allele mining of natural variation and the design of artificial variants**

After identification of genes that are responsible for quantitative traits, 'allele mining' can be performed. This compares the level of function of a particular gene, and its availability for breeding, in two parental varieties.

For example, in the case of *ckx2* alleles, which encode a cytokinin oxidase/dehydrogenase (part **A** in the figure) the variety with higher grain yield, Habataki, has lower levels of expression than one of the standard varieties, Koshihikari, although both alleles encode functional cytokinin oxidase/dehydrogenase. In Habataki, the lower expression results in lower levels of cytokinin-degrading enzymes in the inflorescence meristem, leading to an increase in the number of grains. Hence, a partial loss-of-function allele of *ckx2* in Habataki has been selected in the process of breeding, whereas Koshihikari contains a gain-of-function allele. The expected direction for finding a superior allele leading to better yield is mining of a gene allele with less function. The predicted direction of suitability (indicated by the arrow at the bottom of the graph) was verified by the finding that a high-yielding variety, 5150, with much higher grain yield than Habataki carries a null allele of *ckx2*, this being the most suitable allele for high yield.



In general — in contrast to the simple case as described above — to find the most suitable function among gene alleles, other gene variants with different levels of gene function should be tested in terms of availability for breeding. Possible situations in which conflicts between gene function and availability for breeding affect the overall suitability of an allele are illustrated in part **B** of the figure, which is based on the effects of two initial gene alleles. In the case of pattern **a**, in which two additional gene variants with increased function exist among naturally occurring variation (indicated by brown circles), one has a more advantageous effect on availability for breeding, but a second additional allele with an even greater gain-of-function causes detrimental side-effects with respect to this attribute. Consequently, the former is the most suitable allele, as any artificially designed alleles with increased function are also likely to have negative effects on suitability for breeding (dashed brown line). By contrast, in patterns **b** and **c**, increased gene function among variants that occur within naturally existing variation (alleles represented as yellow and blue circles) does not have a negative effect on availability for breeding. In such cases, 'artificial design' of new variants (indicated by triangles) and introduction of these variants by transgenic approaches should be useful for finding the most suitable alleles, which might lie beyond the limits of natural variation. In pattern **b**, optimal gene function is found among artificially designed alleles. In the case of pattern **c**, the generation of additional gain-of-function alleles is required for optimization (dashed blue line).

This advanced allele mining strategy, which is extended by the artificial design of genes, is potentially powerful for crop improvement. Using this strategy, the limitations of plant species for allele mining by crossing using traditional breeding approaches could also be overcome, for example, by introducing a corresponding gene with a more relevant function from other species, such as a transgene. Such a new allele might be found, for example, by carrying out an *in vitro* assay of the activity of the encoded proteins so that the effects of artificial mutations could be examined more efficiently. Alternatively, advantageous gene variants might be found in wild relatives or other species, the habitats of which feature stressful environments. Once such a superior gene allele is found, homologous recombination might also be useful to introduce small mutations at the specific loci[107]. Thus, a combination of QTL mapping and the transgenic approach is likely to become increasingly important in the near future.

supply and thereby inhibition of respiration. Rice, which has interconnected gas spaces called aerenchyma, is the one of the few crops species that has an ability to germinate and grow in waterlogged soils. However, under complete submergence conditions most rice cultivars cannot survive for more than a week, but a few cultivars, such as FR13A of the *indica* cultivar, can survive up to two weeks. A major QTL, *Submergence1* (*Sub1*), is linked to the submergence tolerance of FR13A[65]. This locus contains a cluster of three genes (*Sub1A*, *Sub1B*, and *Sub1C*) that encode putative ethylene response factors (ERFs)[66]. The responsible gene for submergence tolerance has been identified as *Sub1A*[67]. Among *Sub1* locus haplotypes in 17 *indica* and 4 *japonica* varieties, the tolerance is correlated with the specific allele *Sub1A*-1, with a SNP between this and the intolerant allele. Ectopic expression of *Sub1A*-1 in an intolerant variety increases survival during submergence. Moreover, introgression of the *Sub1* genes into the widely grown Indian variety Swarna, which lacks *Sub1A*, confers strong submergence tolerance without affecting yield, plant height, harvest index, or grain quality indicated by amylose content. Development of submergence-tolerant varieties using the same breeding procedure is at an advanced stage for south Asian countries, and has also been reported in Thailand[68].

In terms of mechanism, the nature of submergence tolerance remains unclear. SUB1A has been proposed to suppress carbohydrate consumption and thereby compromise energy deficit under submergence by inhibition of action of SUB1C, ethylene and GA[69]. However, there has not been as much progress on submergence tolerance by genetic analyses other than *Sub1* studies. Moreover, different mechanisms might control submergence tolerance in crops other than rice. Considering that many crops are sensitive to soil dampness, further investigation is needed to understand the mechanisms of submergence tolerance in these species.

Aluminium tolerance. In highly acidic soils, which constitute up to a half of the world's arable lands, aluminium (Al) toxicity causes a significant reduction in crop production[70]. At low pH levels (<5.5), Al^{3+} ions are solubilized and this inhibits root growth and function, resulting in limitations in water and nutrient uptake. In many plant species, a major mechanism involved in Al tolerance is exudation of organic acids such as malate, citrate and oxalate from the root[70]. These compounds chelate and detoxify Al toxicity in the rhizosphere.

Recent studies have revealed the genes encoding transporters of Al-chelating organic acids that are responsible for Al tolerance. In wheat, *ALMT1* (encoding the Al-activated malate efflux transporter) is likely to correspond to Alt_{BH}, a major QTL for Al tolerance[71,72]. *ALMT1* is constitutively expressed in the root apices, and Al-tolerant and Al-sensitive lines differ in levels of its expression[71,72]. Moreover, transgenic barley expressing *ALMT1* exhibits increased Al tolerance[73]. In sorghum, the gene encoding an Al-activated citrate efflux transporter, MATE, which belongs to the multidrug and toxic compound extrusion (MATE) family, was identified as the major QTL for Al tolerance (Alt_{SB})[74]. Al-tolerant varieties have an enhanced expression of *MATE* specifically in root apices, resulting in increased citrate exudation.

The studies in both wheat and sorghum suggest that a suitable way to confer Al tolerance in a breeding strategy should be to identify and use a higher expression allele of the gene encoding the Al-chelating organic-acid transporter. A similar strategy would be applied to some other crop species by manipulation of the corresponding genes using a transgenic approach. It will also be interesting to see whether combinational enhancement of the efflux of different organic acids — by introduction of the corresponding transporter genes — can be observed and whether this results in even higher levels of tolerance. In rice, however, an increase in malate efflux by the introduction of wheat *ALMT1* seems not to be sufficient to confer Al tolerance[71]. Hence, relatively Al-tolerant species such as rice might have another tolerance mechanism. If so, those species possibly offer additional opportunities for the discovery of genes to further enhance Al tolerance.

Tolerance to toxicity and deficiency of boron. Boron is an essential micronutrient for plant growth and is required for the crosslinking of the cell-wall molecule rhamnogalacturonan II[75,76]. However, excess boron confers toxicity to plants. Because of the narrow range between deficient and toxic concentrations, both limiting and toxic soil concentrations of boron cause major limitations of crop production worldwide[77,78]. Evidence of boron deficiency has been reported in over 80 countries, and it is estimated that around 15 million hectares of lands are treated with boron annually[77]. Areas with high soil boron include dry lands of South Australia, the Middle East, the west coast of Malaysia, the southern coast of Peru and the Andes foothills in northern Chile[78]. Toxicity of boron is difficult to manage agronomically and is often problematic, particularly in equatorial and arid regions, together with salinity[78].

Molecular mechanisms that are used by plants to control boron concentrations have recently been revealed by the identification of boron transporters (FIG. 2). Under physiological pH conditions, boron exists mainly as uncharged boric acid that is permeable through the plasma membrane, allowing passive diffusion that is also facilitated by plasma membrane-located channel proteins, such as maize PIP1 proteins (REF. 79) and *A. thaliana* NIP5-1 (REF. 80), which belong to the group of major intrinsic proteins (MIPs). Expression of *NIP5-1* is upregulated under boron limitation in the root elongation zone and root hair, and mutation in *NIP5-1* causes lower boric-acid uptake in roots and increases sensitivity to boron deficiency[80]. However, it is unclear whether MIPs are the limiting transporters for boron utilization in crop species.

Boron is also transported by active transporters localized in the plasma membrane. BOR1 in *A. thaliana* is the first boron transporter identified in any organism[81]. The *A. thaliana* loss-of-function mutant, *bor1-1*, is sensitive to boron deficiency and is impaired in

Ethylene response factor (ERF). Refers to members of a subfamily of the AP2 transcription factor family, which is unique to the plant lineage. ERF subfamily proteins carry a domain that is conserved in ethylene-responsive element-binding proteins.

Major intrinsic proteins (MIPs). A large protein family, the members of which act as channels in membranes to facilitate passive transport of small polar molecules such as water, glycerol and urea across the membrane.

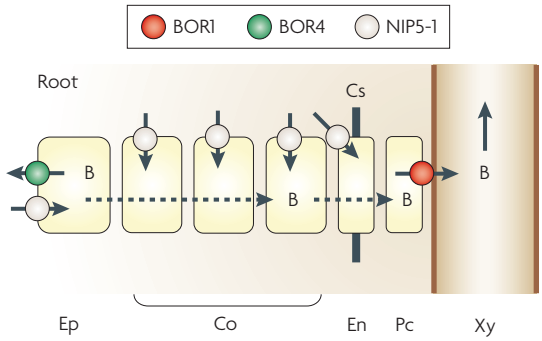


Figure 2 | **Boron transporters.** Transport of boric acid (B) across membranes in *Arabidopsis thaliana* is facilitated by a plasma membrane-located channel protein, NIP5-1, and by cell-type specific efflux transporters (BOR1 and BOR4). NIP5-1 and BOR1 are involved in boron uptake under boron-deficient conditions, whereas the boron-exclusion activity of BOR4 confers tolerance to toxic concentrations of boron. Boron that is taken up into root cells from the soil is moved through a symplastic pathway by plasmodesmata (dashed arrows), whereas apoplastic movement is hindered by the casparian strip (Cs). Co, cortex; En, endodermis; Ep, epidermis; Pc, pericycle; Xy, xylem. This figure is modified, with permission, from REF. 84 © (2007) American Society of Plant Biologists.

root-to-shoot transport of boron by xylem loading. BOR1 has ten putative transmembrane domains, is similar to animal bicarbonate transporters and its expression in yeast results in decreased boron concentrations, indicating that BOR1 is an efflux-type transporter. BOR1 is specifically localized in the pericycle of roots in *A. thaliana*, and mediates boron export from pericycle cells into the root stelar apoplasm against a concentration gradient. Under boron-limiting conditions, overexpression of BOR1 increases both boron translocation to the shoot apex and its root-to-shoot translocation[82]. Under conditions of high boron concentration, BOR1 is transferred from the plasma membrane via the endosomes to the vacuole and then degraded[83]. This is a mechanism to avoid accumulation of toxic levels of boron in shoots. Recently, the rice orthologue of BOR1 has been shown to have a role in boron uptake and xylem uploading[84]. It will be interesting to determine whether the genetic manipulation of transporters in crop species will provide a practical means of protection from boron deficiency.

By contrast, in studies of plant resistance to boron toxicity, a boron-tolerant cultivar of barley — Sahara — accumulated lower levels of boron accumulation in both shoot and root[85]. It was suggested that this was mediated by active efflux pumping from root cells. Consistent with this, high expression levels of *BOR2*, a barley homologue of *BOR1* located at chromosome 4H, correlated with a reduction in boron concentration in roots when using lines that were generated by backcrossing Sahara with a boron-sensitive cultivar, Sloop[86]. QTLs responsible for tolerance to boron toxicity had also been identified in a cross of Sahara and another sensitive variety, Clipper[87]. The major locus affects boron accumulation and leaf symptoms under boron-toxic conditions[88]. By high-resolution mapping of this locus, *Bot1*, a *BOR1* orthologue encoding a functional boron efflux transporter, was identified as the gene responsible for the boron-toxicity tolerance[88]. Compared with Clipper, Sahara contains four times more *Bot1* copies, and 160-fold and 18-fold higher levels of *Bot1* transcripts in roots and leaf blades, respectively. The importance of BOR1-related efflux transporters was further demonstrated by functional analyses of *A. thaliana* BOR4 (REF. 89). In contrast to BOR1, BOR4 is not degraded but is accumulated at increased levels in high-boron conditions. Moreover, BOR4 is localized in the plasma membrane of the distal sites of epidermal cells in the elongation zone of roots, probably enabling the directional export of boron to the soil (FIG. 2). Furthermore, overexpression of BOR4 causes a stronger tolerance to toxic concentrations of boron, concomitant with lower levels of boron accumulation in roots and shoots compared with wild-type plants.

Both the QTL studies in monocot crops and the molecular analyses in *A. thaliana* suggest that improvement of crop productivity in boron-toxic soils should be achievable by the introduction of boron efflux transporter genes with enhanced expression. These results also suggest that cell type-specific regulation of transporter genes is important for effective boron transport. Considering that the mechanism conferring boron tolerance is so conserved across species, it might be possible to manipulate *BOR* genes in many different crop species to achieve the same effect, perhaps even using the same transgene in different species, if driven by suitable promoters. This hypothesis will require careful examination under field conditions.

Salt tolerance. There have been numerous molecular genetic and physiological studies on salt tolerance, mostly in *A. thaliana*[20,53,54,90] (FIG. 3a). Because high concentrations of cytosolic Na^+ are toxic, it is important to eliminate Na^+ from the cytosol by transporters. The Na^+–H^+ exchanger SOS1 facilitates efflux of Na^+ across the plasma membrane, whereas the vacuolar Na^+–H^+ antiporter NHX1 transports cytosolic Na^+ into vacuoles[20,53,54,90]. Overexpression of SOS1, NHX1 and their homologues confers enhanced salt tolerance in many plant species, including rice and maize[54]. The same is true for AVP1, an H^+-pyrophosphatase; interestingly, overexpression of an *AVP1* allele that carries a gain-of-function mutation results in not only enhanced tolerance to salt and drought, but also an increased root biomass and phosphorus nutrition[91–93].

At whole-plant levels, it is thought that the balance of Na^+ and K^+ ions (the cellular Na^+/K^+ ratio) is also important for salt tolerance, especially in cereal crops[94,95]. Supporting this theory, the *Kna1* locus was identified as contributing to a lower Na^+/K^+ ratio and to higher salt tolerance in bread wheat[94] and, in wild relatives of barley with strong salt tolerance, accumulation of Na^+ in leaf blades following salt stress were at strikingly lower levels compared with the cultivated barley[96]. In rice, several research groups have dissected the QTLs

responsible for variations in Na^+ and K^+ content[97]. Using the salt tolerant *indica* rice variety Nona Bokra and the salt sensitive *japonica* variety Koshihikari, Lin *et al.* mapped a major QTL for shoot K^+ content in seedlings, *Skc1*, which was revealed to encode a HKT-type transporter, SKC1 (also known as HKT8 and later designated as HKT1;5)[98–100]. SKC1 is the selective transporter for Na^+, which is localized at the plasma membrane and is preferentially expressed in the parenchyma cells surrounding the xylem vessels (FIG. 3b). The nearly isogenic line *NIL(SKC1)*, which contains the salt-tolerant *SKC1* allele of Nona Bokra within the genetic background of salt-sensitive Koshihikari, was demonstrated to confer salt tolerance at the seedling stage. Under salt stress, *NIL(SKC1)* exhibits higher K^+ content and lower Na^+ content in the shoot than the Koshihikari variety. In roots, both Na^+ and K^+ content are the same in the two lines under both normal and stress conditions, although expression of *SKC1* is upregulated by salt stress in roots but not in shoots. In xylem saps but not phloem saps, *NIL(SKC1)* has more K^+ and less Na^+ than in Koshihikari under salt stress, although no difference was observed under normal conditions.

Based on these results, it is suggested that SKC1 contributes to Na^+ and K^+ translocation between roots and shoots, possibly by Na^+ unloading from xylem, thereby affecting the Na^+/K^+ ratio in the shoots. There are no substantial differences in the expression pattern between *NIL(SKC1)* and Koshihikari. However, when expressed in the *Xenopus laevis* oocytes, SKC1 from Nona Bokra exhibits higher sodium-transporting activity than SKC1 from Koshihikari. Thus, the four-amino-acid difference between the encoded SKC1 proteins, which is located in the loops between the putative transmembrane domains, is likely to be responsible for the functional difference between the two varieties.

In durum wheat, the QTL *Nax1* has been studied as a genetic component that confers lower Na^+ and higher K^+ concentrations in the leaf blade[101]. Using NILs, *Nax1* was shown to have a role conferring salt tolerance through higher levels of Na^+ exclusion from the xylem in the roots and leaf sheath, thereby reducing Na^+ concentration

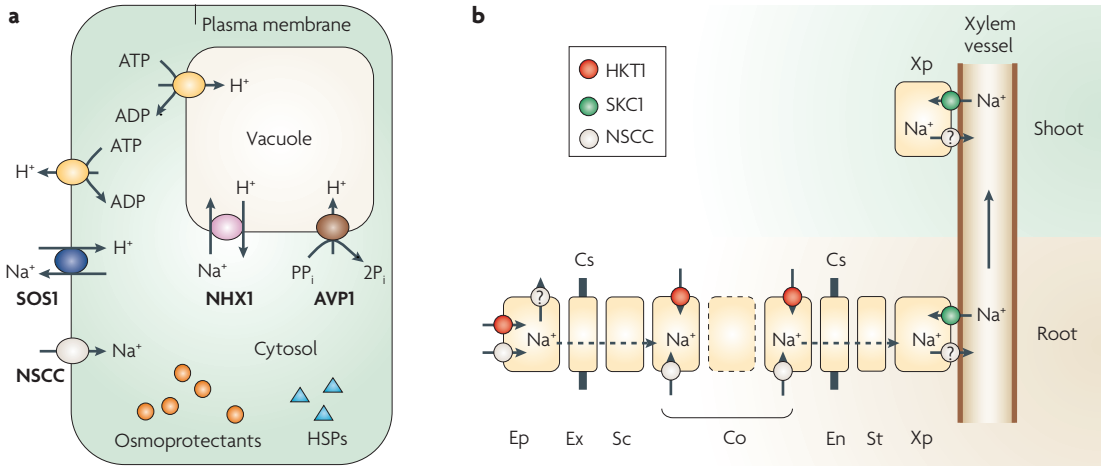


Figure 3 | **Mechanistic models of salt tolerance. a** | Cellular responses to Na^+ toxicity. It is thought that Na^+ is passively transported into the cytosol by non-selective cation channels (NSCCs)[108]. To decrease cytosolic Na^+ concentration, two mechanisms operate. One is to exclude Na^+ across the plasma membrane. SOS1, a Na^+–H^+ exchanger located at the plasma membrane, is the only transporter identified in plants that carries out this function. Another mechanism is to compartmentalize Na^+ into vacuoles, where Na^+ is less toxic. This is performed by the vacuolar Na^+–H^+ antiporter, NHX1. Both transporter activities require an H^+ gradient across the membranes, which is generated either by a plasma membrane H^+-ATPase or by a vacuolar H^+-ATPase (yellow ovals), and by vacuolar H^+-pyrophosphatase (AVP1). Accumulation of osmoprotectants (for example, betaine, proline and sugar alcohols) and heat shock proteins (HSPs) are also induced by salt stress. Overexpression of SOS1, NHX1, and AVP1, as well as HSPs or proteins for the biosynthesis of osmoprotectants, has been reported to enhance salt tolerance in several plant species. For more details, see REFS 53,54 for reviews. **b** | Transport of sodium ions in rice. Na^+, which is taken up into root cells from soils, presumably by NSCCs, is moved by the symplastic pathway through plasmodesmata, whereas apoplastic movement of Na^+ is hindered by the casparian strip (Cs). For long-distance movement through xylem vessels in an apoplastic manner, Na^+ is uploaded from root xylem parenchyma cells (Xp) and unloaded by leaf xylem parenchyma cells. SKC1, a rice HKT transporter (also known as OsHKT1;5 and previously named OsHKT8), is proposed to take up Na^+ from xylem vessels into xylem parenchyma. The role of this HKT transporter is also supported by recent studies of an *Arabidopsis thaliana* homologue, HKT1;1. Another HKT-type transporter, HKT1 (also known as OsHKT2;1)[109] is involved in Na^+ uptake in the epidermis (Ep) and cortex (Co) in roots only in soil conditions with low concentrations of K^+ and Na^+. This uptake is downregulated in the presence of K^+ and Na^+, thus it is negligible in stress conditions involving salinity. Export of Na^+ from epidermal cells and xylem parenchyma is mediated by SOS1 in *A. thaliana*, and possibly by the SOS1 homologue in rice. For more detailed information, see REF. 90 for a review. Ex, exodermis; Sc; sclerenchyma cells; En, endodermis; St, stele. Dashed line, breakage of cortex cells generating aerenchyma. Part **a** is modified, with permission, from REF. 54 © (2005) Cell Press. Part **b** is modified, with permission, from *Nature Genetics* REF. 110 © (2005) Macmillan Publishers Ltd.

Table 1 | **Genes responsible for abiotic stress tolerance and yield improvement in crops identified by QTL analysis**

Trait	Crop	Gene (and proportion of variance explained)	Encoded protein	Difference between the parental varieties	Nature of allele suitable for use in improvement	Refs
Fruit size	Tomato	*fw2.2* (30%)	RAS-related protein	Expression level	Low expression	45–47
Grain size (weight)	Rice	*gs3* (20%)	Transmembrane protein	Nonsense mutation	Loss of function	48
Grain size (length)	Rice	*gs3* (55%)	Transmembrane protein	Nonsense mutation	Loss of function	48
Grain size (width)	Rice	*gw2* (5–8%)	RING-type ubiquitin E3 ligase3	Premature stop	Loss of function	49, 50
Grain number	Rice	*Gn1a* (44%)	Cytokinin oxidase/ dehydrogenase	Expression level	Loss of function	51
Submergence tolerance	Rice	*Sub1A* (70%)	ERF-related factor	Presence or absence	Gain of function	65, 67
Aluminium tolerance	Wheat	*ALMT1* (80%)	Malate efflux transporter	Expression level	High expression	72
Aluminium tolerance	Sorghum	*MATE* (80%)	Citrate efflux transporter	Expression level	High expression	74
Boron toxicity tolerance	Barley	*Bot1* (34%)	Boron efflux transporter	Expression level	High expression	86–88
Salt tolerance (shoot K^+ concentration)	Rice	*SKC1* (40%)	HKT-type Na^+ transporter	Amino-acid substitution	Gain of function	98, 99
Salt tolerance (low Na^+ in leaves)	Wheat	*Nax1* (38%)	HKT-type Na^+ transporter	Expression level	High expression	103

in the leaf blades[102]. By comparative mapping of wheat and rice chromosomes, *HKT7-A2*, encoding a sodium transporter, was suggested to be a strong candidate gene for *Nax1* (REF. 103). *HKT7-A2* is expressed in both roots and leaf sheaths, but not in leaf blades, of salt-tolerant wheat line 149 and *Triticum monococcum* C68-101, whereas no transcripts are detected in the salt-sensitive Tamaroi variant. This possibly accounts for the decreased levels of Na^+ in leaf blades in the tolerant lines, by a mechanism similar to that proposed from the study of SKC1 in rice.

It is still ambiguous, however, to what extent SKC1-related transporters account for salt tolerance in general, given that three major QTLs other than *SKC1* contribute to seedling survivability under salt stress in rice[98]. Each of these QTLs accounts for 14% to 18% of the total phenotypic variance. Thus, identification of the genes for such QTLs and more genetic studies in monocot crops should be important for a better understanding of the salt-tolerance mechanism at the seedling stage.

As in the case of boron efflux transporters, manipulation of the Na^+ transporter gene in a cell type-specific manner should be effective to confer salt tolerance in many crops. Such fine regulation would also be beneficial to minimize potential side-effects. Using a stress-induced promoter is an alternative way to achieve this purpose, as exemplified previously[104]. To transfer the obtained knowledge to a more practical application, it is important to consider genetic interactions and genotype-by-environment interactions. It will be essential to directly compare the ability of various genes to confer salt tolerance, using the cultivars on regional demands — through either introgression of the major QTLs or through manipulation of genes. Combinational introduction of beneficial genes by breeding and transgenic approaches should also be evaluated to use their potential abilities more effectively.

Conclusions and future directions

Traditional molecular genetic studies have contributed to our understanding of the underlying biology involved in abiotic stress tolerance and yield control, whereas QTL studies have revealed genetic components that are available for their improvement in existing varieties. There has been good progress in gene identification by QTL mapping for traits, including yield and tolerance to soil stresses (TABLE 1), some of which provide additional clues for improvement of specific traits in agriculture. Association mapping and selection screens are potentially useful approaches for future mapping studies, but have so far been performed only in maize and *A. thaliana*. To use these methods, and QTL mapping, more effectively, it will be important to develop applicable resources, including large-scale collections of cultivars, landraces or germplasm lines as well as inbred lines derived from them, with extensive information about SNPs and other genetic markers. Also, better knowledge of population structures is important for better design of association studies and for data interpretation. Studies that combine QTL mapping and association analysis might prove more successful than either strategy alone. An ongoing effort to enable such studies is the development of a large-scale maize-mapping population, comprising 5,000 recombinant inbred lines derived from crossing each of 25 diverse inbred lines to an standard line, B73, in conjunction with validation of thousands of SNPs (for details, see the Panzea web site)[22]. Association studies of candidate genes, the functions of which are revealed by QTL mapping and/or other approaches, will be especially useful for allele mining.

Some initial insights have been provided into the architecture of traits relevant to crop improvement, which gives important information for strategies to use the genetic components that are identified. For example,

Recombinant inbred line (RIL). A progeny line carrying dispersed homozygous segments of a parental chromosome, formed after several selfed generations of an F2 line.

for grain yield in rice, several major QTLs account for large portions of the phenotypic variation. The introduction of such major QTLs provides an efficient strategy for crop improvement, and introducing combinations of different beneficial loci by QTL pyramiding is a promising approach. Such a strategy is much less powerful if a trait is controlled by many QTLs with small effects. Importantly, therefore, the success of the QTL-based approach largely depends on the choice of parental varieties at the starting point. A variety carrying a marked improvement in an advantageous trait is most desirable to be used in combination with a standard variety that has been selected by traditional breeding programmes. However, so far, QTL studies have often been started with varieties that are chosen on the basis of the availability of genetic markers, rather than the advantageousness of traits, leading to a reduced chance of finding major QTLs with large effects. This is in part because of the limitation of available sequence information or, more specifically, marker information. Recent and future advances in sequence technologies and polymorphism detection will lead to improvements in this area and will facilitate more trait-oriented studies.

Another important issue in QTL studies will be to understand the nature of heterosis, or hybrid vigour, which results in the phenotypic superiority of a hybrid over parental lines and thus has already been used for practical breeding[105]. An approach to assess overdominant effects on heterosis, using introgression lines of tomato, has recently been reported[106]. It will also be essential to investigate complexities that arise owing to genetic and genotype-by-environment interactions, in order to better understand the genetic architecture of complex agronomic traits and explore how allelic variation in hybrids might contribute to plant flexibility in responding to environmental variation — particularly the variation associated with climate change.

Studies carried out so far have also provided insights into the extent to which genes implicated in advantageous traits are likely to be useful across different crop species. For improving tolerance to soil stresses, introduction of specific ion transporters is likely to be an effective strategy. Because these transporters are highly conserved, manipulation of the same gene could also be used across species. This is in contrast to the gene for a regulator protein involved in submergence tolerance in rice, which seems to be effective only in a species-specific manner. Further improvement using transporters should be achieved if superior gene alleles could be found in natural variations and in induced genetic variations (for example, by chemical mutagenesis), or by artificial design.

Similar to QTL pyramiding, the introduction of combinations of different beneficial genes into an elite variety through the transgenic approach is also likely to provide an important direction in crop improvement (we can call this 'transgenic pyramiding'). Because stress conditions, such as salinity and drought, vary depending on local climates and geographical features, the fine tuning of gene activities will be required for practical application in each area. For optimization in respective conditions, allele mining and gene pyramiding (by either hybridization or gene manipulation) will be useful to produce a series of plants carrying different combinations of advantageous genes at different levels of activity. Selecting the optimal combinations among these variations and evaluating the specific trade-offs, in terms of biological adaptation, productivity and economic value, promises to provide solutions to pre-existing abiotic stresses as well as prospective environmental changes.

1. Rosegrant, M. W. & Cline, S. A. Global food security: challenges and policies. *Science* **302**, 1917–1919 (2003).
2. Stocking, M. A. Tropical soils and food security: the next 50 years. *Science* **302**, 1356–1359 (2003).
3. Schmidhuber, J. & Tubiello, F. N. Global food security under climate change. *Proc. Natl Acad. Sci. USA* **104**, 19703–19708 (2007).
4. Brown, M. E. & Funk, C. C. Food security under climate change. *Science* **319**, 580–581 (2008).
5. Chrstensen, J. H. *et al.* in *Climate change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S. *et al.*) 848–940 (Cambridge University Press, UK and New York, USA, 2007).
6. Meehl, G. A. *et al.* in *Climate change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S. *et al.*) 748–845 (Cambridge University Press, UK and New York, USA, 2007).
7. Lobell, D. B. *et al.* Prioritizing climate change adaptation needs for food security in 2030. *Science* **319**, 607–610 (2008).
8. McCouch, S. R. & Doerge, R. W. QTL mapping in rice. *Trends Genet.* **11**, 482–487 (1995).
9. Mackay, T. F. The genetic architecture of quantitative traits. *Annu. Rev. Genet.* **35**, 303–339 (2001).
10. Ashikari, M. & Matsuoka, M. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends Plant Sci.* **11**, 344–350 (2006).
11. Alonso, J. M. & Ecker, J. R. Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in *Arabidopsis*. *Nature Rev. Genet.* **7**, 524–536 (2006).
12. Jung, K. H., An, G. & Ronald, P. C. Towards a better bowl of rice: assigning function to tens of thousands of rice genes. *Nature Rev. Genet.* **9**, 91–101 (2008).
13. Candela, H. & Hake, S. The art and design of genetic screens: maize. *Nature Rev. Genet.* **9**, 192–203 (2008).
14. Ichikawa, T. *et al.* The FOX hunting system: an alternative gain-of-function gene hunting technique. *Plant J.* **48**, 974–985 (2006).
15. Nakamura, H. *et al.* A genome-wide gain-of function analysis of rice genes using the FOX-hunting system. *Plant Mol. Biol.* **65**, 357–371 (2007).
16. International Rice Genome Sequencing Project. The map-based sequence of the rice genome. *Nature* **436**, 793–800 (2005).
17. Devos, K. M. Updating the 'crop circle'. *Curr. Opin. Plant Biol.* **8**, 155–162 (2005).
18. Kling, J. The search for a sequencing thoroughbred. *Nature Biotech.* **23**, 1333–1335 (2005).
19. Liang, C. *et al.* Gramene: a growing plant comparative genomics resource. *Nucleic Acids Res.* **36**, D947–D953 (2008).
20. Zhu, J. K. Plant salt tolerance. *Trends Plant Sci.* **6**, 66–71 (2001).
21. Yamaguchi-Shinozaki, K. & Shinozaki, K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* **57**, 781–803 (2006).
22. Yu, J. & Buckler, E. S. Genetic association mapping and genome organization of maize. *Curr. Opin. Biotechnol.* **17**, 155–160 (2006).
23. Flint-Garcia, S. A., Thornsberry, J. M. & Buckler, E. S. Structure of linkage disequilibrium in plants, *Annu. Rev. Plant Biol.* **54**, 357–374 (2003).
24. Kim, S. *et al.* Recombination and linkage disequilibrium in *Arabidopsis thaliana*. *Nature Genet.* **39**, 1151–1155 (2007).
25. Hirschhorn, J. N. & Daly, M. J. Genome-wide association studies for common diseases and complex traits. *Nature Rev. Genet.* **6**, 95–108 (2005).
26. Aranzana, M. J. *et al.* Genome-wide association mapping in *Arabidopsis* identifies previously known flowering time and pathogen resistance genes. *PLoS Genet.* **1**, 531–539 (2005).
27. Zhao, K. *et al.* An *Arabidopsis* example of association mapping in structured samples. *PLoS Genet.* **3**, 71–82 (2007).
This paper, as well as references 24 and 26, discusses the feasibility of genome-wide association mapping in plants.
28. Yu, J. *et al.* A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genet.* **38**, 203–208 (2006).
This paper reports a new statistical method to handle population structure for association mapping.
29. Buckler, E. & Gore, M. An *Arabidopsis* haplotype map takes root. *Nature Genet.* **39**, 1056–1057 (2007).
30. Thornsberry, J. M., *et al. Dwarf8* polymorphisms associate with variation in flowering time. *Nature Genet.* **28**, 286–289 (2001).

31. Palaisa, K. A., Morgante, M., Williams, M. & Rafalski, A. Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci. *Plant Cell* **15**, 1795–1806 (2003).
32. Wong, J. C., Lambert, R. J., Wurtzel, E. T. & Rocheford, T. R. QTL and candidate genes phytoene synthase and zeta-carotene desaturase associated with the accumulation of carotenoids in maize. *Theor. Appl. Genet.* **108**, 349–359 (2004).
33. Harjes, C. E. *et al.* Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science* **319**, 330–333 (2008).
This paper is an example of a successful study using combinational approaches, with association analysis, QTL mapping and chemical mutagenesis revealing that the variation at the *lycE* locus has major effects on carotenoid and provitamin A composition in maize grain.
34. Wright, S. I. *et al.* The effects of artificial selection on the maize genome. *Science* **308**, 1310–1314 (2005).
35. Yamasaki, M. *et al.* A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. *Plant Cell* **17**, 2859–2872 (2005).
36. Yamasaki, M., Wright, S. I. & McMullen, M. D. Genomic screening for artificial selection during domestication and improvement in maize. *Ann. Bot.* **100**, 967–973 (2007).
37. Doebley, J. F., Gaut, B. S. & Smith, B. D. The molecular genetics of crop domestication. *Cell* **127**, 1309–1321 (2006).
38. Hamblin, M. T. *et al.* Challenges of detecting directional selection after a bottleneck: lessons from *Sorghum bicolor*. *Genetics.* **173**, 953–964 (2006).
39. Hedden, P. The genes of the Green Revolution. *Trends Genet.* **19**, 5–9 (2003).
40. Nakagawa, M., Shimamoto, K. & Kyozuka, J. Overexpression of *RCN1* and *RCN2*, rice *TERMINAL FLOWER 1/CENTRORADIALIS* homologs, confers delay of phase transition and altered panicle morphology in rice. *Plant J.* **29**, 743–750 (2002).
41. Suzaki, T. *et al.* The gene *FLORAL ORGAN NUMBER1* regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. *Development* **131**, 5649–5657 (2004).
42. Kurakawa, T. *et al.* Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652–655 (2007).
43. Bommert, P., Satoh-Nagasawa, N., Jackson, D. & Hirano, H. Y. Genetics and evolution of inflorescence and flower development in grasses. *Plant Cell Physiol.* **46**, 69–78 (2005).
44. Kellogg, E. A. Floral displays: genetic control of grass inflorescences. *Curr. Opin. Plant Biol.* **10**, 26–31 (2007).
45. Frary, A. *et al. fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. *Science* **289**, 85–88 (2000).
46. Cong, B., Liu, J. & Tanksley S. D. Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. *Proc. Natl Acad. Sci. USA* **99**, 13606–13611 (2002).
47. Liu, J., Cong, B. & Tanksley, S. D. Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned quantitative trait locus *fw2.2* controls fruit size. *Plant Physiol.* **132**, 292–299 (2003).
48. Fan, C. *et al. GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor. Appl. Genet.* **112**, 1164–1171 (2006).
49. Yoon, D. B. *et al.* Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa japonica* cultivar Hwaseongbyeo. *Theor. Appl. Genet.* **112**, 1052–1062 (2006).
50. Song, X. J., Huang, W., Shi, M., Zhu, M. Z. & Lin, H. X. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genet.* **39**, 623–630 (2007).
51. Ashikari, M. *et al.* Cytokinin oxidase regulates rice grain production. *Science* **309**, 741–745 (2005).
This paper provides evidence that levels of the phytohormone cytokinin in the inflorescence affect the branching pattern of the panicle, and thus the grain yield, of rice. A QTL pyramiding method in rice is also demonstrated.
52. Xing, Y. Z., Tang, W. J., Xue, W. Y., Xu, C. G. & Zhang, Q. Fine mapping of a major quantitative trait loci, *qSSP7*, controlling the number of spikelets per panicle as a single Mendelian factor in rice. *Theor. Appl. Genet.* **116**, 789–796 (2008).
53. Zhu, J. K. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* **53**, 247–273 (2002).
54. Yamaguchi, T. & Blumwald, E. Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci.* **10**, 615–620 (2005).
55. Valliyodan, B. & Nguyen, H. T. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol.* **9**, 189–195 (2006).
56. Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K. & Shinozaki, K. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. Biotechnol.* **17**, 113–122 (2006).
57. Nelson, D. E. *et al.* Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc. Natl Acad. Sci. USA* **104**, 16450–16455 (2007).
This paper reports a good example to show that the identification of a transcription factor in model plants can be applied for crop improvement in the field.
58. Sanchez, A. C., Subudhi, P. K., Rosenow, D. T. & Nguyen, H. T. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant Mol. Biol.* **48**, 713–726 (2002).
59. Rivero, R. M. *et al.* Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc. Natl Acad. Sci. USA* **104**, 19631–19636 (2007).
60. Harris, K. *et al.* Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *J. Exp. Bot.* **58**, 327–338 (2007).
61. Landi, P. *et al. Root-ABA1* QTL affects root lodging, grain yield, and other agronomic traits in maize grown under well-watered and water-stressed conditions. *J. Exp. Bot.* **58**, 319–326 (2007).
62. Tuberosa, R. & Salvi, S. Genomics-based approaches to improve drought tolerance of crops. *Trends Plant Sci.* **11**, 405–412 (2006).
63. Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. & Atlin, G. A Large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci.* **47**, 507–516 (2007).
64. Kumar, R., Venuprasad, R. & Atlin, G. N. Genetic analysis of rainfed lowland rice drought tolerance under naturally occurring stress in eastern India: heritability and QTL effects. *Field Crops Res.* **103**, 42–52 (2007).
65. Xu, K., Xu, X., Ronald, P. C. & Mackill, D. J. A high-resolution linkage map of the vicinity of the rice submergence tolerance locus *Sub1*. *Mol. Gen. Genet.* **263**, 681–689 (2000).
66. Gutterson, N. & Reuber, T. L. Regulation of disease resistance pathways by AP2/ERF transcription factors. *Curr. Opin. Plant Biol.* **7**, 465–471 (2004).
67. Xu, K. *et al. Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* **442**, 705–708 (2006).
This study revealed the responsible gene allele for submergence tolerance among natural variations at the *Sub1* locus in rice.
68. Siangliw, M., Toojinda, T., Tragoonrung, S. & Vanavichit, A. Thai jasmine rice carrying *QTLch9* (*Sub*QTL) is submergence tolerant. *Ann. Bot.* **91**, 255–261 (2003).
69. Fukao, T., Xu, K., Ronald, P. C. & Bailey-Serres, J. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* **18**, 2021–2034 (2006).
70. Kochian, L. V., Hoekenga, O. A. & Pineros, M. A. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.* **55**, 459–493 (2004).
71. Sasaki, T. *et al.* A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* **37**, 645–653 (2004).
72. Raman, H. *et al.* Molecular characterization and mapping of *ALMT1*, the aluminium-tolerance gene of bread wheat (*Triticum aestivum* L.). *Genome* **48**, 781–791 (2005).
73. Delhaize, E. *et al.* Engineering high-level aluminum tolerance in barley with the *ALMT1* gene. *Proc. Natl Acad. Sci. USA* **101**, 15249–15254 (2004).
74. Magalhaes, J. V. *et al.* A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nature Genet.* **39**, 1156–1161 (2007).
This paper, and references 71–73, show the gene(s) responsible for organic-acid exudation, which confers aluminium tolerance in crops.
75. Matoh, T., Kawaguchi, S. & Kobayashi, M. Ubiquity of a borate-rhamnogalacturonan II complex in the cell walls of higher plants. *Plant Cell Physiol.* **37**, 636–640 (1996).
76. O'Neill, M. A., Eberhard, S., Albersheim, P. & Darvill, A. G. Requirement of borate cross-linking of cell wall rhamnogalacturonan II for *Arabidopsis* growth. *Science* **294**, 846–849 (2001).
77. Shorrocks, V. M. The occurrence and correction of boron deficiency. *Plant Soil* **193**, 121–148 (1997).
78. Nable, R. O., Banuelos, G. S. & Paull, J. G. Boron toxicity. *Plant Soil* **193**, 181 (1997).
79. Dordas, C., Chrispeels, M. J. & Brown, P. H. Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. *Plant Physiol.* **124**, 1349–1362 (2000).
80. Takano, J. *et al.* The *Arabidopsis* major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* **18**, 1498–1509 (2006).
81. Takano, J. *et al. Arabidopsis* boron transporter for xylem loading. *Nature* **420**, 337–340 (2002).
This is the first report of a boron transporter, which led to subsequent findings of related boron transporter genes, which are responsible for tolerance to boron toxicity (see references 86, 88–89).
82. Miwa, K., Takano, J. & Fujiwara, T. Improvement of seed yields under boron-limiting conditions through overexpression of BOR1, a boron transporter for xylem loading, in *Arabidopsis thaliana*. *Plant J.* **46**, 1084–1091 (2006).
83. Takano, J., Miwa, K., Yuan, L., von Wirén, N. & Fujiwara, T. Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. *Proc. Natl Acad. Sci. USA* **102**, 12276–12281 (2005).
84. Nakagawa, Y. *et al.* Cell-type specificity of the expression of Os *BOR1*, a rice efflux boron transporter gene, is regulated in response to boron availability for efficient boron uptake and xylem loading. *Plant Cell* **19**, 2624–2635 (2007).
85. Hayes, J. E. & Reid, R. J. Boron tolerance in barley is mediated by efflux of boron from the roots. *Plant Physiol.* **136**, 3376–3382 (2004).
86. Reid, R. Identification of boron transporter genes likely to be responsible for tolerance to boron toxicity in wheat and barley. *Plant Cell Physiol.* **48**, 1673–1678 (2007).
87. Jefferies, S. P. *et al.* Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* **98**, 1293–1303 (1999).
88. Sutton, T. *et al.* Boron-toxicity tolerance in barley arising from efflux transporter amplification. *Science* **318**, 1446–1449 (2007).
89. Miwa, K. *et al.* Plants tolerant of high boron levels. *Science* **318**, 1417 (2007).
90. Apse, M. P. & Blumwald, E. Na^+ transport in plants. *FEBS Lett.* **581**, 2247–2254 (2007).
91. Gaxiola, R. A. *et al.* Drought- and salt-tolerant plants result from overexpression of the AVP1 H^+ pump. *Proc. Natl Acad. Sci. USA* **98**, 11444–11449 (2001).
92. Park, S. *et al.* Up-regulation of a H^+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. *Proc. Natl Acad. Sci. USA* **102**, 18830–18835 (2005).
93. Yang, H. *et al.* Enhanced phosphorus nutrition in monocots and dicots over-expressing a phosphorus-responsive type I H^+-pyrophosphatase. *Plant Biotechnol. J.* **5**, 735–745 (2007).
94. Dubcovsky, J., Santa Maria, G., Epstein, E., Luo, M. C. & Dvořák, J. Mapping of the K^+/Na^+ discrimination locus *Kna1* in wheat. *Theor. Appl. Genet.* **2**, 448–454 (1996).
95. Maathuis, F. J. M. & Amtmann, A. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Ann. Bot.* **84**, 123–133 (1999).
96. Garthwaite, A. J., von Bothmer, R. & Colmer, T. D. Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na^+ and Cl^- into the shoots. *J. Exp. Bot.* **56**, 2365–2378 (2005).
97. Sahi, C., Singh, A., Kumar, K. Blumwald, E. & Grover, A. Salt stress response in rice: genetics, molecular biology, and comparative genomics. *Funct. Integr. Genomics* **6**, 263–284 (2006).

98. Lin, H. X. *et al.* QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet.* **108**, 253–260 (2004).
99. Ren, Z. H. *et al.* A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genet.* **37**, 1141–1146 (2005).
This is the first study to reveal a gene that is responsible for salt tolerance in monocot cereals by a forward genetics approach. The authors propose that the sodium transporter is involved in long-distance translocation of Na^+, which is important for salt tolerance in plants.
100. Platten, J. D. *et al.* Nomenclature for HKT transporters, key determinants of plant salinity tolerance. *Trends Plant Sci.* **11**, 372–374 (2006).
101. Lindsay, M. P., Lagudah, E. S., Hare, R. A. & Munns, R. (2004). A locus for sodium exclusion (*Nax1*), a trait for salt tolerance, mapped in durum wheat. *Funct. Plant Biol.* **31**, 1105–1114 (2004).
102. James, R. A., Davenport, R. J. & Munns, R. Physiological characterization of two genes for Na^+ exclusion in durum wheat, *Nax1* and *Nax2*. *Plant Physiol.* **142**, 1537–1547 (2006).
103. Huang, S. *et al.* A sodium transporter (HKT7) is a candidate for *Nax1*, a gene for salt tolerance in durum wheat. *Plant Physiol.* **142**, 1718–1727 (2006).
104. Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. & Shinozaki, K. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnol.* **17**, 287–291 (1999).
105. Lippman, Z. B. & Zamir D. Heterosis: revisiting the magic. *Trends Genet.* **23**, 60–66 (2007).
106. Semel, Y. *et al.* Overdominant quantitative trait loci for yield and fitness in tomato. *Proc. Natl Acad. Sci. USA* **103**, 12981–12986 (2006).
107. Terada, R., Johzuka-Hisatomi, Y., Saitoh, M., Asao, H. & Iida, S. Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics. *Plant Physiol.* **144**, 846–856 (2007).
This report and previous studies by the same group have showed the advance in homologous recombination in rice.
108. Demidchik, V. & Maathuis, F. J. Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. *New Phytol.* **175**, 387–404 (2007).
109. Horie, T. *et al.* Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. *EMBO J.* **26**, 3003–3014 (2007).
110. Rus, A. M., Bressan, R. A. & Hasegana, P. M. Unraveling salt tolerance in crops. *Nature Genet.* **37**, 1029–1030 (2005).

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ALMT1 | *Bot1* | *FRI* | *PSY1* | *RPM1* | *RPS2* | *RPS5*
Gramene: http://www.gramene.org
ckx2 | *Gn1a* | *gs3* | *gw2* | *HKT7-A2* | OsCKX2 | *sd1* | *Sub1*
UniProtKB: http://ca.expasy.org/sprot
AVP1 | BOR1 | NFYB1 | NHX1 | NIP5-1 | SKC1 | SOS1

FURTHER INFORMATION

Shin Takeda's homepage: http://www.agr.nagoya-u.ac.jp/~nubs/index-e.html
DOE Joint Genome Institute (JGI): http://www.jgi.doe.gov/sequencing/cspseqplans2006.html
Maizesequence: http://www.maizesequence.org/index.html
Maizegenome: http://www.maizegenome.org
Panzea: http://www.panzea.org
Phytozome: http://www.phytozome.net/sorghum
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