

Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability

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Abstract | Crop yield reduction as a consequence of increasingly severe climatic events threatens global food security. Genetic loci that ensure productivity in challenging environments exist within the germplasm of crops, their wild relatives and species that are adapted to extreme environments. Selective breeding for the combination of beneficial loci in germplasm has improved yields in diverse environments throughout the history of agriculture. An effective new paradigm is the targeted identification of specific genetic determinants of stress adaptation that have evolved in nature and their precise introgression into elite varieties. These loci are often associated with distinct regulation or function, duplication and/or neofunctionalization of genes that maintain plant homeostasis.

Germplasm

A collection (either wild or cultivated) of diverse genetic material.

Plasticity

The ability of an organism to modify its phenotype (for example, metabolism or development) in response to the environment.

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A twenty-first century challenge is the production of sufficient food to meet population demands despite reductions in the quantity and quality of arable land and water and increasingly variable weather patterns that are associated with climate change. Crop losses due to extremes in the environment have risen steadily over the past several decades^{1,2}, and climate models predict an increased incidence of floods³, droughts⁴ and extreme temperatures^{5,6}. Integrated climate change and crop production models project declines in the yields of major crops such as corn, wheat and rice with serious ramifications on global food production this century^{7–9}. Despite the progressive increase in the production of major crops through germplasm development and agronomic practices since the 1960s, susceptibility to climate variability has risen owing to higher sowing densities, which increase competition for water and nutrients¹⁰. The rapid development and adoption of climate-resilient crop genotypes is imperative to ensure global food security.

Plants successfully occupy diverse and variable environments owing to plasticity in cellular metabolism, physiology and development. Abiotic stress refers to suboptimal climatic and/or edaphic conditions that adversely affect cellular homeostasis and that ultimately impair growth and fitness. For terrestrial species, these stresses include water surplus or deficit, ion toxicity (for example, Al³⁺, Cl[–], Cd²⁺, Fe²⁺ and Na⁺), ion deficiency (for example, Fe³⁺, N, P, S and Zn²⁺), temperature extremes and tropospheric ozone (O₃) (TABLE 1). Stress episodes can be classified as transient stress (for example,

midday high temperatures) or chronic stress (for example, high Na⁺ in sodic soils), and the timing of stress relative to the diurnal cycle and the developmental age of the organism determines the impact on viability and yield. Stress during early vegetative stages slows growth (cell division and expansion) but may not greatly reduce the yield of seed crops, whereas stress during reproductive development can considerably diminish productivity¹¹. Abiotic stresses often occur in combination (for example, heat and drought or heat and O₃) or in succession (for example, flooding followed by drought), so yield maintenance in variable environments can require the enhancement of multiple mechanisms. Agricultural productivity will rise if reduction in yield caused by abiotic stress is minimized.

Plants exhibit stress tolerance or stress avoidance through acclimation and adaptation mechanisms that have evolved through natural selection. Following stress recognition, regulatory responses ultimately re-establish cellular and organismal homeostasis or reduce episodic shock effects. Traits that enable higher yields under an abiotic stress often display functional conservation across species when plants are adapted to similar environments (for example, drought tolerance via deeper rooting) and can reflect derivation from monophyletic origin or convergent evolution. A cache of genetic diversity that is accessible for the improvement of yield stability resides in the germplasm of crops and their wild relatives that grow in wide geographical and climatic ranges. The use of this natural variation for crop improvement

Table 1 | **Abiotic stresses, their constraints and effective survival strategies**

| Stress | Constraint | Tolerance and survival strategy* | Transient solution | Chronic solution | Refs |
|-----------------------------------|---|--|--|---|-------|
| Flooding | Reduced energy owing to lower photosynthesis rate and/or low O ₂ levels | Energy conservation or expenditure | Growth quiescence | <ul style="list-style-type: none"> • Rapid growth for avoidance • Aerenchyma for aeration | 1 |
| Drought | Low water potential | <ul style="list-style-type: none"> • Limited water loss • Improved water uptake | <ul style="list-style-type: none"> • Hydrotropism • Reduced transpiration | <ul style="list-style-type: none"> • Deeper roots • Reduction of leaf area • Adjusted osmotic status | 33 |
| Salinity | Elevated salt levels (for example, NaCl) cause ion cytotoxicity and reduce osmotic potential | <ul style="list-style-type: none"> • Reduced root ion uptake • Vacuolar ion compartmentalization • Osmotic adjustment | <ul style="list-style-type: none"> • Limited ion movement to transpiration stream • Reduced shoot growth | <ul style="list-style-type: none"> • Limited root ion flux to shoots • Vacuolar ion compartmentalization in shoot cells | 71,72 |
| Ion toxicity | Cytotoxicity | <ul style="list-style-type: none"> • Limited uptake • Vacuolar ion compartmentalization | <ul style="list-style-type: none"> • Efflux of organic acids to apoplast and immobilization of ions by chelation • Intracellular chelation | <ul style="list-style-type: none"> • Compartmentalization of ions (for example, vacuole and apoplast) • Efflux of organic ions to chelate toxic ions in soil | 84,95 |
| Ion deficiency | Inadequate nutrient acquisition | Enhanced uptake by transporters and developmental adaptations | <ul style="list-style-type: none"> • Transport protein induction and activation • Reduced growth | <ul style="list-style-type: none"> • Transport protein function • Root sensing and architecture remodelling for acquisition • Partitioning for storage | 99 |
| Low and sub-freezing temperatures | <ul style="list-style-type: none"> • Membrane damage • Low water potential | <ul style="list-style-type: none"> • Low-temperature acclimation • Induction of stress protection genes | <ul style="list-style-type: none"> • Acclimation • Dormancy • Osmoprotection | <ul style="list-style-type: none"> • Acclimation and dormancy • Altered membrane composition • Increased compatible solutes | 105 |
| High temperature | <ul style="list-style-type: none"> • Reduced photosynthesis • Reduced transpiration • Impaired cellular function | Maintenance of membrane function and reproductive viability | <ul style="list-style-type: none"> • Leaf cooling • Molecular chaperones | <ul style="list-style-type: none"> • Altered membrane composition • Molecular chaperones | 5 |
| Ozone (>120 nL) | <ul style="list-style-type: none"> • Reduced photosynthesis • ROS | Increased capacity to control ROS | <ul style="list-style-type: none"> • Stomatal closure • Elevated antioxidant capacity | Elevated antioxidant capacity | 154 |

ROS, reactive oxygen species. *Depending on the species and developmental state, the effective survival strategy may be tolerance (for example, metabolic acclimation for survival) or avoidance (for example, escape of drought by deeper rooting). Avoidance strategies may be constitutive or stress-induced evolutionary adaptations.

Edaphic conditions

Conditions related to soil, including physical (for example, texture and drainage) and chemical (for example, pH and elemental content) properties.

Transient stress

An aberrant change in the environment lasting for a short period of time — for example, high vapour pressure deficit at midday and temporary flooding.

Chronic stress

A suboptimal environmental condition lasting for a substantial portion of the life cycle of the plant — for example, salt accumulation in fertilized fields and season-long water stress.

has generally been without knowledge of specific causal genes and associated biological mechanisms. Currently, key genetic determinants can be identified through quantitative trait locus (QTL) mapping, association mapping and screening by recurrent selection (reviewed in REF. 12). The recent detailed molecular characterization of QTLs and the associated genes that aid stress survival has enabled the more expedient transfer of genes into modern crop germplasm by following molecular markers associated with the key genes during breeding. In many instances, advancement in the identification of the gene that determines the trait has led to insights into the specific biological mechanism, and the translation of knowledge to other species. Complementing the use of existing genetic diversity are investigations into the molecular, cellular and physiological processes that are integral to the environmental adaptation and acclimation of model species, which can accelerate the recognition of key loci in crops and can also foster biotechnologically engineered solutions.

In this Review, we highlight the utilization of effective stress adaptation loci that confer traits that provide yield stability in the field under environmental extremes involving water, temperature and ions (TABLE 2). We also summarize engineered solutions for abiotic stress that have been validated in the field (see [Supplementary](#)

[information S1 \(table\)](#)). Two major classes of genes predominate as tolerance determinants: transcription factors and transporters. The underlying genetic differences associated with these effective loci include allelic variation in coding and regulatory sequences, copy number variation (CNV) and/or gene neofunctionalization following duplication. We focus on genetic determinants that have been introgressed into elite lines, tested under field conditions and are the targets of breeding programmes or have already been adopted by farmers.

Rescuing rice from floods

Submergence tolerance in rain-irrigated rice. Asian cultivated rice (*Oryza sativa* L.) was domesticated from wild populations of *Oryza rufipogon* and *Oryza nivara* through selection for desirable grain size and number and for reduced seed dispersal. Progenitor traits that were beneficial in local ecosystems, such as submergence survival, were retained by selection in local landraces, particularly those of the *aus* subgroup of *O. sativa* ssp. *indica*¹³. An example of this is the Indian landrace Dhalputtia (FR13A), the source of the *SUBMERGENCE1* (*SUB1*) locus that confers submergence tolerance by restricting underwater elongation growth, which is a quiescence strategy¹⁴ (FIG. 1a). The ability of FR13A to survive more than 2 weeks of complete submergence contrasts with the

Table 2 | **Gene-to-field examples of effective genetic variation in abiotic stress survival strategies***

| Stress | Gene and locus | Species | Donor | Genetic basis [†] | Gene product function | Functional mechanisms | Refs |
|---|--|--|--|----------------------------|--|--|-------------------------------|
| Flooding | <i>SUB1A</i> | <i>Oryza sativa</i> | Landrace | NF; CNV; AV | Ethylene-responsive TF (ERF-VII) | Ethylene-promoted inhibition of GA-mediated elongation growth; energy conservation | 14,17, 18 |
| | <i>SK1</i> and <i>SK2</i> | <i>O. sativa</i> | Landrace | P/A; CNV | Ethylene-responsive TF (ERF-VII) | Ethylene-promoted, GA-mediated elongation of stem internodes | 24 |
| Drought | <i>DRO1</i> | <i>O. sativa</i> | Tolerant genotype | AV | Unknown function | Enhanced deep rooting; auxin-regulated asymmetric root growth | 41,42 |
| Saline soil | <i>HKT1;5-A</i> at the <i>Nax2</i> locus | <i>Triticum turgidum</i> ssp. <i>durum</i> | <i>Triticum monococcum</i> | P/A | Na ⁺ transporter | Root Na ⁺ exclusion from xylem vessels | 76,77 |
| | <i>TaHKT1;5</i> | <i>Triticum aestivum</i> | <i>T. aestivum</i> D genome (origin <i>Aegilops tauschii</i>) | AV | Na ⁺ transporter | Higher K ⁺ /Na ⁺ in expanding and young leaves | 78 |
| | <i>HKT1;5</i> | <i>O. sativa</i> ssp. <i>japonica</i> | Tolerant <i>O. sativa</i> ssp. <i>indica</i> | AV | Na ⁺ transporter | Shoot K ⁺ /Na ⁺ homeostasis | 80 |
| High Al ³⁺ | <i>SbMATE</i> at the <i>Alt_{SB}</i> locus | <i>Sorghum bicolor</i> | Tolerant genotype | AV | Root Al ³⁺ -activated citrate efflux transporter | Citrate chelation of Al ³⁺ detoxifies root zone | 90 |
| | <i>MATE1</i> | <i>Zea mays</i> | Tolerant genotype | CNV; AV | Root Al ³⁺ -activated citrate efflux transporter | Citrate chelation of Al ³⁺ detoxifies root zone | 91 |
| | <i>ALMT</i> | <i>T. aestivum</i> | <i>T. aestivum</i> (origin probably <i>A. tauschii</i>) | CNV; AV | Root Al ³⁺ -activated malate efflux transporter | Malate chelation of Al ³⁺ detoxifies root zone | 85,86, 152 |
| | <i>NRAT1</i> | <i>O. sativa</i> | Tolerant genotype | AV | Root Al ³⁺ -activated (<i>NRAT1</i> expression) Nrap Al ³⁺ transporter (plasma membrane influx) | Intracellular Al ³⁺ influx decreases toxic build-up in the cell wall and presumably facilitates vacuolar compartmentalization | 92 |
| High B | <i>Bot1</i> | <i>Hordeum vulgare</i> | Landrace | CNV; AV | Root B efflux transporter | Restricts net B accumulation | 96 |
| | <i>Bot-B5b</i> | <i>T. aestivum</i> | Landrace | AV | Root B efflux transporter | Restricts net B accumulation | 98 |
| Low P _i | <i>PSTOL1</i> at the <i>Pup1</i> locus | <i>O. sativa</i> | Landrace | P/A; AV | Protein kinase | Enhanced crown root development for improved P _i acquisition | 13,102 |
| Low Fe ³⁺ and Zn ²⁺ | <i>NAM-B1</i> | <i>T. turgidum</i> ssp. <i>durum</i> | Related subspecies | AV | NAC TF | Accelerates senescence and nutrient remobilization to grain | 155 |
| Low temperature | <i>VRN1</i> at the <i>FR1</i> locus | <i>T. aestivum</i> | Wild relative | CNV; AV | MADS box TF | Regulation of vernalization; low-temperature-induced <i>CBF</i> and regulation activation; freezing tolerance | 108,109, 110,112 |
| | | <i>H. vulgare</i> | Tolerant genotype | | | | |
| | <i>CBFs</i> at the <i>FR2</i> locus | <i>T. aestivum</i> | Wild relative | CNV; AV | <i>CBFs</i> (TFs) | <i>CBF</i> copy number-dependent expression and regulon activation; freezing tolerance | 109,110, 111,114, 15,116, 117 |

ALMT, Al³⁺-activated malate transporter; AV, allelic variation; CNV, copy number variation; *DRO1*, *DEEPER ROOTING1*; *FR*, *FROST RESISTANCE*; *HKT1*, *HIGH-AFFINITY K⁺ TRANSPORTER1*; *MATE*, multidrug and toxic compound extrusion; NF, neofunctionalization; *NRAT1*, *NRAMP Al³⁺ TRANSPORTER 1*; P/A, present or absent locus; *PSTOL1*, *PHOSPHATE STARVATION TOLERANCE1*; *Pup1*, *Phosphorus uptake 1*; *SK*, *SNORKEL*; *SUB1*, *SUBMERGENCE1*; TF, transcription factor; *VRN1*, *VERNALIZATION1*. *Examples limited to genes or loci from plant germplasm with demonstrated effectiveness in the field. [Supplementary information S1 \(table\)](#) provides other examples of transgenes that have conferred stress survival and yield stability to crops in the field.

Stress tolerance

The ability to maintain metabolic function, growth and/or yield despite the presence of an abiotic stress, achieved via mechanisms such as ion homeostasis and osmotic adjustment.

Stress avoidance

Mechanisms whereby an organism is able to alter or halt the effect of a stress by mechanisms such as ion exclusion, stomatal closure and leaf movements.

Acclimation

A short-term (typically within a generation) modification at the genetic, cellular and/or organismal levels that allows a plant to survive an aberrant change in the surrounding environment.

Adaptation

A long-term (typically over generations) modification at the genetic level that allows a plant to survive a change in its environment. The ability to quickly acclimate (for example, to transient stress) can be an adaptive trait.

Monophyletic origin

Organisms descended from a common ancestor.

Convergent evolution

Evolution via independent paths to a similar outcome, such as a solution to an abiotic constraint.

Yield stability

The maintenance of crop yield, despite suboptimal growth conditions, such as the presence of an abiotic stress.

Quantitative trait locus

(QTL). A region of chromosome that determines a trait that is quantitative in nature.

Allelic variation

Distinction in the coding sequence or regulatory regions of an allele (form of a gene at the same genetic locus).

semi-dwarf Green Revolution varieties that thrive in shallow paddies but that die within a week of full inundation (TABLE 1). Fine mapping of *SUB1* identified a genetically varied chromosomal region, which harbours two or three genes encoding ethylene-response factor (ERF) DNA-binding proteins¹⁴ (FIG. 1b), arising from gene CNV and neofunctionalization that apparently predates domestication¹⁵. Of the three ERFs at the *SUB1* locus (*SUB1A*, *SUB1B* and *SUB1C*), only the strongly submergence-induced allele *SUB1A-1* is limited to submergence-tolerant landraces^{14,16}. The transgenic overexpression of this gene in a flooding-intolerant *O. sativa* ssp. *japonica* variety confirmed that it is a robust submergence tolerance determinant¹⁴.

Investigation of the functional role of the *SUB1* genes determined that, as floodwaters rise, the gaseous phytohormone ethylene that is trapped within cells promotes *SUB1A-1* expression¹⁷ (FIG. 1c), which enhances the accumulation of two transcription factors that impede the response to the phytohormone gibberellin (GA): SLENDER RICE 1 (SLR1) and SLR1-LIKE 1 (SLRL1)¹⁸. The outcome is a restriction of cell elongation and the less rapid consumption of energy reserves^{17–19}. Upon de-submergence, genotypes with *SUB1A-1* incur less tissue damage from reactive oxygen species (ROS) and the dehydration that is associated with re-aeration²⁰. Even when leaf tissue loss is substantial, the maintenance of tiller meristems enables *SUB1A-1* genotypes to resume development when floodwaters subside.

Submergence tolerance conferred by *SUB1A-1* has been expeditiously introgressed by marker-assisted breeding into popular high-yielding varieties that are grown in flood-prone regions of Asia and Africa²¹. The productivity and quality of these Sub1 varieties is indistinguishable from that of the parental cultivars under non-submerged conditions^{14,21,22}; based on field trials and farmers' experience, *SUB1A-1* significantly buffers yield losses without affecting other valued traits²³ (FIG. 1d,e). Currently, an estimated 10 million farmers have access to Sub1 varieties (A. Ismail, International Rice Research Institute (IRRI), Philippines; personal communication, 2015).

Escape from submergence by rice grown in deep water.

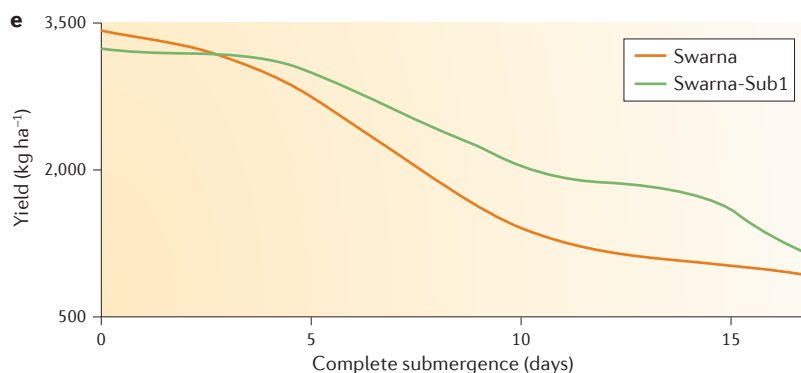
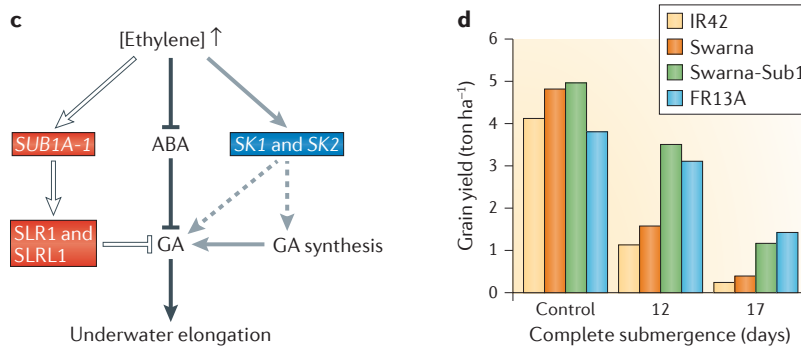
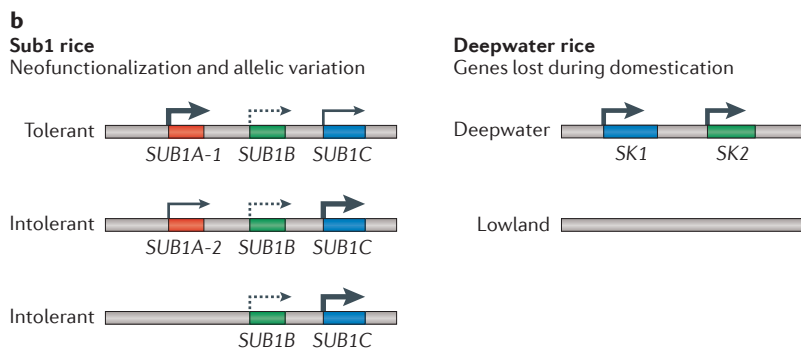
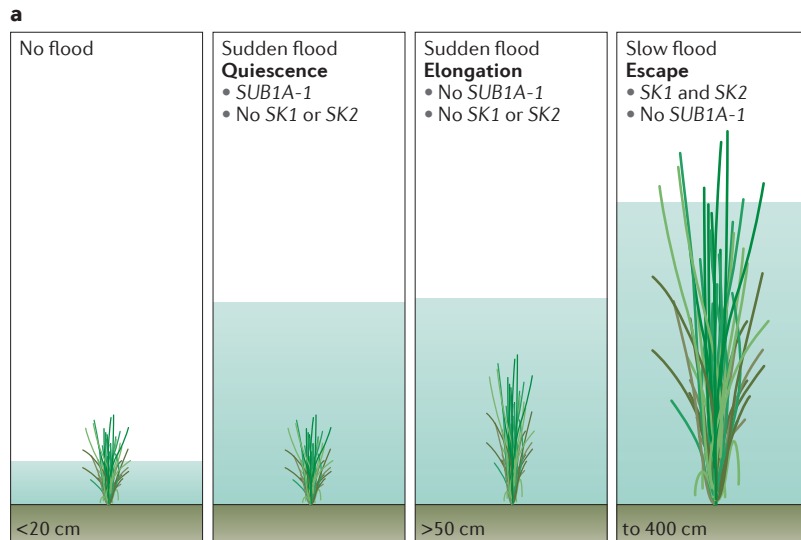
An effective flooding avoidance strategy is displayed by the 'deepwater' or 'floating' landraces of rice that avoid complete submergence in waters that rise to heights of 4 m during their life cycle by the investment of energy into enhanced elongation of submerged stems (FIG. 1a). These porous structures are connected to root aerenchyma, which allow gas exchange between non-submerged and submerged tissue. Three QTLs from the Thai deepwater accession C9285 are sufficient to confer the underwater elongation response in flooding-intolerant *O. sativa* ssp. *japonica* rice²⁴. One of these is the *SNORKEL* (SK) locus, which also displays gene CNV — in this case, 0–2 ethylene-upregulated ERFs (SK1 and SK2) that are closely related to the *SUB1* genes (FIG. 1a,b). The SK proteins stimulate, rather than repress, GA-promoted elongation growth, specifically in the sixth and higher stem internodes. The enhancement of

Figure 1 | **Flooding survival in rice.** **a** | Strategies used by rice to survive different types of floods are shown. **b** | In submergence-tolerant (Sub1) rice, the determinant transcription factor gene allele *SUBMERGENCE1A-1* (*SUB1A-1*) is strongly upregulated by submergence compared with the *SUB1A-2* allele^{14,17}. The paralogue *SUB1C* is also regulated by submergence. In deepwater rice, the rapid underwater elongation genes *SNORKEL1* (SK1) and SK2 are absent in lowland varieties and are closely related to the *SUB1* genes²⁴. The thickness of the right-angled arrows represents submergence-induced changes in transcript abundance, with the dashed arrows indicating even lower increases. **c** | *SUB1A-1* confers tolerance through the quiescence of growth. SK1 and SK2 enable avoidance by escape (underwater elongation). The ethylene, abscisic acid (ABA) and gibberellin (GA) underwater elongation pathway is restricted by SUB1A (which enhances the accumulation of the SLENDER RICE 1 (SLR1) and SLR1-LIKE 1 (SLRL1) transcription factors that repress GA responsiveness) but is promoted by SK1 and SK2 (REFS 17, 18, 24, 25). **d** | A controlled wet season field trial from 2008 is shown. IR42 and Swarna are high-yielding lowland varieties; Swarna-Sub1 has the *SUB1* locus from FR13A²³. **e** | Data from the 2011 wet season from 133 farmers' fields comparing near-isogenic lines are shown²². Introgression of the *SUB1* locus from FR13A into the widely grown Swarna variety reduces yield loss following prolonged submergence. Part **d** reprinted from *Field Crops Res.*, 113, Singh, S., Mackill, D. J. & Ismail, A. M., Responses of SUB1 rice introgression lines to submergence in the field: yield and grain quality, 12–23, Copyright (2009), with permission from Elsevier. Part **e** reproduced from REF. 22, Nature Publishing Group.

GA responsiveness, combined with increased biosynthesis of GA, enables starch catabolism for stem elongation in deepwater rice²⁵ (FIG. 1c). SKs are found in the genomes of accessions of wild *O. rufipogon* from Asia and *Oryza glumaepatula* from South America, indicating that an ancient genomic region of *Oryza* was lost during the establishment of rice grown in shallow paddies but was safeguarded by farmers cultivating a deepwater ecosystem.

Paths to improvement of flooding tolerance in other crops.

With the exception of rice, most crops are extremely sensitive to periods of waterlogging or to partial-to-complete submergence. The discovery of the SUB1 and SK1/2-like ERFs (of the ERF-VII subclass) led to the recognition that ERF-VIIs function as pivotal regulators of metabolic acclimation during submergence and hypoxia in *Arabidopsis thaliana*¹. The accumulation of these transcription factors is conditional and involves a conserved proteasome-mediated degradation pathway that is regulated by oxygen availability^{26,27}. As ERF-VII-driven gene upregulation in response to submergence or low oxygen stress occurs in diverse species, the manipulation of their accumulation and activity may prove to be an avenue for yield stability under varied flooding regimes in other crops, especially as the escape and quiescence strategies are recognized as effective survival strategies in rice, as well as in wild dicot species²⁸.



The aeration of waterlogged cereal crop roots is facilitated by aerenchyma and a suberized hypodermis that limits the radial diffusion of oxygen out of the root and the formation of adventitious roots with both of these features. Successful movement of loci associated with these traits in wild *Hordeum marinum* and *Zea nicaraguensis* to wheat²⁹ and maize^{30,31}, respectively, illustrates the potential to harness adaptation loci from wild relatives for the improvement of waterlogging tolerance.

Solutions for yield stability under drought

Insufficient soil moisture has complex ramifications, mostly owing to the disruption of the soil–root–plant–atmosphere water potential gradient, resulting in ionic and hyperosmotic constraints on cells (TABLE 1). Breeding for drought tolerance or avoidance has proved challenging, at least partly because tolerance mechanisms are often environment-specific, and screening methods that integrate the multiple spatial and temporal variations that are relevant to this stress are difficult to establish^{32,33}. In addition, drought survival is a trait with strong evolutionary selection pressure. Therefore, there are typically many drought survival loci that together impart tolerance in crop plants³⁴, often with each locus accounting for a relatively small proportion of phenotypic variation^{35,36}. Consequentially, strong genotype × environment plasticity reduces the effectiveness of individual loci in multiple environments^{37–39}. However, the identification of loci that favourably influence drought tolerance across multiple populations and environments can reduce the number of target loci for downstream characterization⁴⁰.

Effective root traits. An example of a drought tolerance trait with a potentially wide application is the establishment of deep roots that can access moisture reserves, an avoidance strategy. A breeding population derived from the widely grown shallow-rooting IR64 rice and the deep-rooting Kinandang Patong landrace identified the *DEEPER ROOTING 1 (DRO1)* QTL that can provide drought yield stability^{41,42} (FIG. 2a,b). A one-nucleotide deletion in the coding sequence of the IR64 allele of *DRO1 (DRO1-ir)* results in a truncated protein and a reduced root angle of curvature in response to gravity; whereas the Kinandang Patong allele, *DRO1-kp*, promotes asymmetric cell elongation in the root tip region, causing downward growth (FIG. 2c). Introgression of *DRO1-kp* into IR64 results in a deep-rooting phenotype in which high yields are maintained under prolonged soil water deficit (FIG. 2d). The deep-rooting phenotype also enhances nutrient uptake and yield under non-drought conditions in the field⁴³, illustrating a tolerance locus that also provides enhanced yield in the absence of stress. Beneficial loci for root traits that should enhance drought tolerance are also known for maize⁴⁴, wheat⁴⁵, sorghum⁴⁶, barley⁴⁷ and chickpea⁴⁸.

Regulation of abscisic acid perception and response to improve drought tolerance. The phytohormone abscisic acid (ABA) has a crucial role in the control

Copy number variation

(CNV). Regions of chromosome that differ because of duplication or deletion of DNA that usually includes complete genes. CNV results in distinct haplotypes at a chromosomal region and is often associated with higher or lower expression owing to gene copy number duplication or deletion, respectively.

Neofunctionalization

Evolution of a duplicated gene that results in the distinct function and/or regulation of a formerly paralogous gene or protein.

of stomatal aperture to reduce transpiration⁴⁹ and in the induction of genes that enable drought acclimation across plant species^{50,51}. Despite detailed knowledge of ABA metabolism, signalling and response mechanisms, there are limited examples of their successful manipulation to improve drought stress tolerance in crops (see Supplementary information S1 (table)). This may change through the characterization of ABA receptors. ABA binding to REGULATORY COMPONENT OF ABA RECEPTOR 1 (RCAR1) was found to be necessary for ABA-mediated inhibition of type 2C protein phosphatases (PP2Cs) that negatively regulate ABA responses in *A. thaliana*⁵². Concurrently, a chemical genomics approach identified the small sulphonamide pyrabactin as a potent ABA agonist, acting through PYRABACTIN RESISTANCE 1 (PYR1). This led to the recognition of 13 related *PYR1-Like* (PYL) genes, including *RCAR*⁵³. When bound to ABA, the PYR1 and PYL receptors competitively inhibit the ability of PP2C to dephosphorylate SnRK2 (SNF1-related kinase), thereby activating ABA-dependent signalling^{54,55}. With an increase in dehydration-induced ABA accumulation, the PYR/PYL–PP2C–SnRK2

module activates guard cell anion channels, leading to plasma membrane depolarization and stomatal closure^{50,51,56,57}. This pathway also upregulates genes that facilitate water-deficit acclimation and tolerance^{56,58}. Both redundancy and specificity in *A. thaliana* PYR/PYL function is evident from their spatial expression, oligomeric states and preferential activation by specific synthetic sulphonamides^{53,55,59}.

The constitutive overexpression of ABA receptors improves drought tolerance^{59–61} but may negatively affect yield under non-stress conditions⁶². This suggests that the precise regulation of the activity of individual or multiple receptors will be required for field-level tolerance. An additional possibility for managing drought response involves reengineering of the ABA receptor. Modification of the ligand-binding pocket of *A. thaliana* PYR1 to bind at nanomolar sensitivity to a non-herbicidal agrichemical applied as a foliar spray was recently used to induce ABA receptor activity and to enhance the drought tolerance of *A. thaliana* and tomato⁶³. This dissection and manipulation of ABA perception, which is also crucial for salt and cold tolerance, provides new opportunities for plant protection.

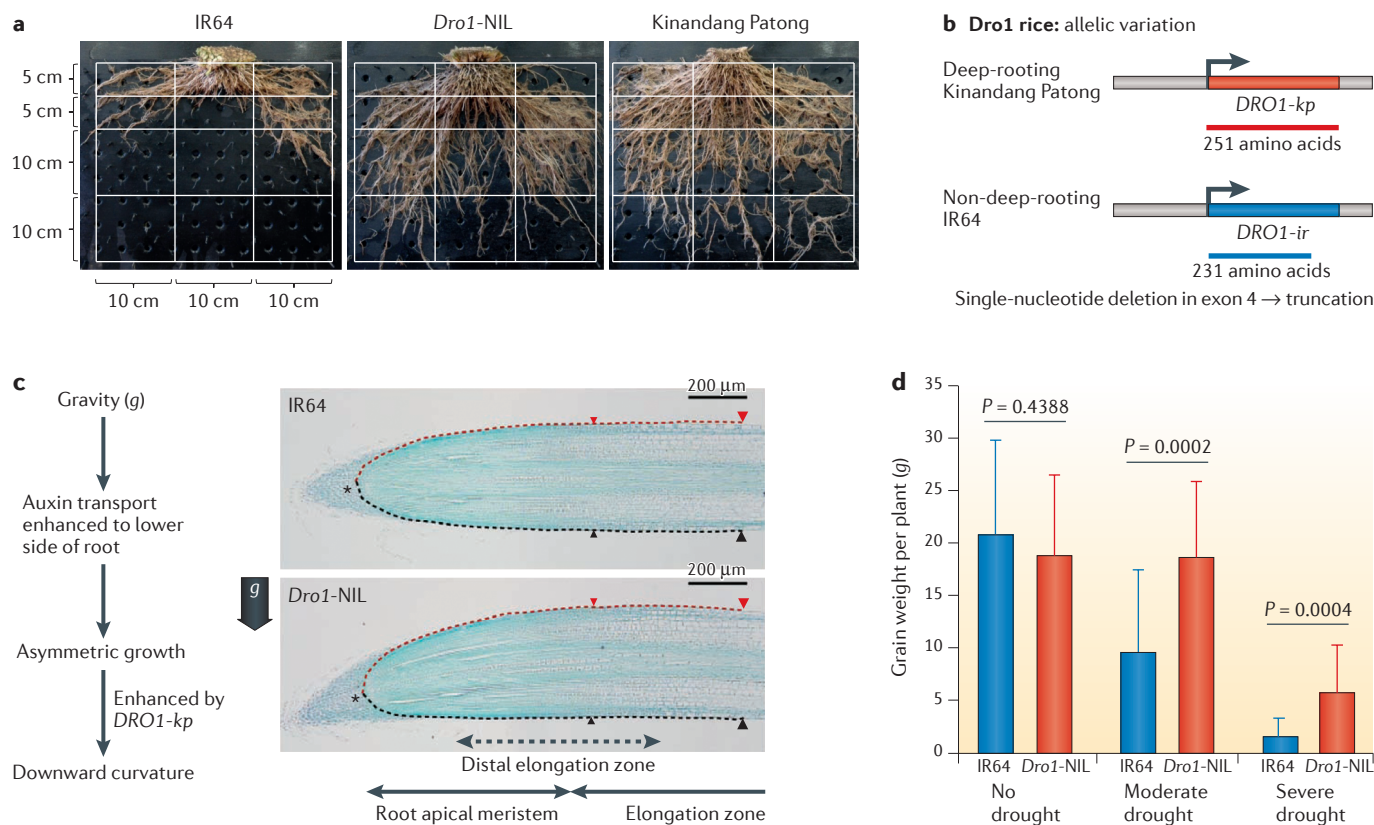


Figure 2 | Deeper rooting in rice for drought tolerance. **a** | The deeper rooting 1 (*Dro1*)-NIL (near-isogenic line) is IR64 with the Kinandang Patong allele (*DRO1*-kp) introgressed by breeding. **b** | Allelic variation at *DRO1* is responsible for variation in root depth and drought tolerance. Accumulation of the transcript of the IR64 allele of *DRO1* (*DRO1*-ir) in the root tip region is lower than that of *DRO1*-kp, and it encodes a truncated *DRO1* protein. **c** | *DRO1*-kp affects auxin-mediated

downward root growth that is stimulated by gravity. Dashed lines indicate outer perimeters from quiescent centres (asterisks) to elongation zones (large arrowheads) on the upper (red) and lower (black) side of roots. **d** | Yield stability observed under moderate and severe drought in the deeper rooting *Dro1*-NIL is shown. Error bars represent standard deviation. Photos in parts **a** and **c** and graph in part **d** are reproduced from REF. 42, Nature Publishing Group.

Multiple mechanisms for enhanced drought tolerance. Given the diverse effects of osmotic stress on plants, it is not surprising that a number of traits and/or determinants for mechanisms other than deep rooting and the regulation of ABA responses have been manipulated to improve drought tolerance. Loci that enable a stay-green phenotype — the maintenance of functional green leaf area during grain maturation through the regulation of cytokinin and ethylene metabolism and the amelioration of ROS — are important tools in cereal breeding programmes^{64,65}, and transgenic studies have established the importance of this trait for drought tolerance in a number of species (see Supplementary information S1 (table)). Basic research has identified other key signalling and regulatory genes that have been manipulated in transgenic plants to provide measurable improvement of drought tolerance³³. For example, the expression of a bacterial RNA-binding protein in maize is sufficient to improve yield stability under drought⁶⁶, and engineered expression of a range of transcription factors, transporters and phytohormones show promise in the field (see Supplementary information S1 (table)). Often, transgenes require cell-specific or environment-specific regulatory elements to obtain drought tolerance without a yield penalty^{67,68}.

Na⁺ homeostasis for salt tolerance

Both natural events and human activity have shaped the worldwide salinity problem; it affects more than 20% of present-day agriculture and continues to intensify owing to fresh water supply depletion that is beyond biogeochemical recycling capacity⁶⁹ and to the increased use of recycled irrigation water⁷⁰. Saline soils lead to elevated leaf cellular Na⁺ and Cl⁻ concentrations that negatively affect crop yield, first by inhibiting cell expansion and photosynthetic activity, and later by cytotoxic effects that accelerate leaf senescence, further reducing carbon assimilation and partitioning to reproductive structures⁷¹. Therefore, conserved salt tolerance mechanisms that control net Na⁺ uptake across the plasma membrane (intracellular influx) and tonoplast (vacuolar and pre-vacuolar compartmentalization) of both root and shoot cells help to minimize cytosolic and organellar ion toxicity, maintain K⁺/Na⁺ homeostasis and facilitate vacuolar osmotic adjustment for turgor maintenance^{69,71–74} (TABLE 2; see Supplementary information S1 (table)).

Allelic variation for sodium transporters. Studies of model plants identified *HKT1* (*HIGH-AFFINITY K⁺ TRANSPORTER1*), *SOS1* (*SALT OVERLY SENSITIVE1*) and *NHX* (*Na⁺/H⁺ EXCHANGER*) as crucial determinants of cellular Na⁺ homeostasis, with *HKT1* and *SOS1* controlling net flux across the plasma membrane and *NHX* controlling movement across the tonoplast membrane into the vacuole^{69,71–73}. More recently, it has become evident that *HKT1* loci and alleles are causal determinants for cereal crop salt tolerance in the field (FIG. 3A; TABLE 2). *HKT1;5* is a plasma membrane-localized Na⁺ transporter that partitions Na⁺ from root xylem vessels to adjacent parenchyma cells and perhaps

into the cortex^{69,71}. Reducing net xylem vessel loading of Na⁺ minimizes transpirational flux into the shoot^{69,75}. Commercial durum varieties (*Triticum turgidum* ssp. *durum*) lack *HKT1;5* (FIG. 3B) and are salt sensitive because of high concentrations of leaf Na⁺ and disturbed leaf K⁺/Na⁺ homeostasis⁷⁶. Introgression of the *Nax2* locus (containing *TmHKT1;5-A*) from the wild wheat relative *Triticum monococcum* into durum wheat substantially reduced leaf Na⁺ concentration owing to effective root xylem vessel exclusion, leading to enhanced grain yield by 25% on saline soils^{76,77}. *Nax2* material has been distributed to ~30 wheat breeding programmes worldwide for introgression into local high-yielding lines (R. Munns, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia; personal communication, 2015). Introgression of *Nax1* (containing *TmHKT1;4-A*) also reduced leaf Na⁺ concentration owing to Na⁺ partitioning from xylem vessels into leaf sheaths. Salt tolerance of modern hexaploid bread wheat (AABB genomes from *T. turgidum* and DD genome from *Aegilops tauschii*) is linked to the *Kna1* locus⁷⁸ (harbouring *TaHKT1;5D*⁷⁹) contributed by *A. tauschii*. *TaHKT1;5D* has neofunctionalized from the *A. tauschii* gene *AtHKT1;5D* during hybridization to have NaCl-induced expression (FIG. 3C). The *TaHKT1;5D* allele benefit is associated with reduced leaf Na⁺ concentration and higher fitness of hexaploid *Triticum aestivum* relative to *T. turgidum* under salt stress⁷⁹.

Distinctions in salt tolerance in rice seedlings are connected with allelic variation at *OsHKT1;5* (previously known as *SKC1*) (FIG. 3D). Some *OsHKT1;5* alleles effectively maintain shoot K⁺/Na⁺ homeostasis owing to specific amino acid variations that enhance K⁺ over Na⁺ transport into root xylem sap, that alter protein transmembrane stability or that alter phosphorylation that affects function⁸⁰. This gene lies within the *Saltol* QTL that was initially described from the Pokkali landrace as providing significant seedling salt tolerance in the field and subsequently directed into breeding programmes⁸¹. Systematic evaluation of *OsHKT1;5* allelic variation and tissue Na⁺ and K⁺ concentrations indicates that *OsHKT1;5* and additional as yet uncharacterized genetic determinants function together to alleviate damage from Na⁺ in *O. sativa* and *Oryza glaberrima* seedlings⁸². K⁺/Na⁺ homeostasis and salt tolerance have also been linked to *HKT1* paralogues in tomato⁸³, further highlighting evolutionary variation in this transporter.

Solutions for aluminium and boron toxicity

In addition to Na⁺, other soil elements may be essential (such as Al³⁺, B, Fe³⁺ and Zn²⁺) or non-essential (such as Cd²⁺) for plant growth, but they may also be toxic in excess, thus necessitating restrictions to their cellular accumulation. Al³⁺, the predominant form of aluminium in acid soils (pH <5.5), readily accumulates in cells because of the electrochemical potential across the plasma membrane and, in excess, it can disturb basic cellular functions and ultimately reduce yield⁷⁸. Substantial allelic variation and CNV exist in crop germplasm for Al³⁺ tolerance, and they have been exploited to enhance

Introgressed

Pertaining to the transfer of a genetic determinant from a donor genotype to a recipient genotype by repeated backcross hybridization. It can be accelerated by the use of molecular markers for the donor chromosomal region, as well as markers surrounding that region and the other chromosomes of the recipient genotype.

Landraces

Locally adapted varieties used by farmers.

Quiescence

A reduction in growth associated with the conservation of energy reserves.

Aerenchyma

Hollow conduits that form within roots and stems to facilitate the exchange of gases. There are multiple processes of aerenchyma development.

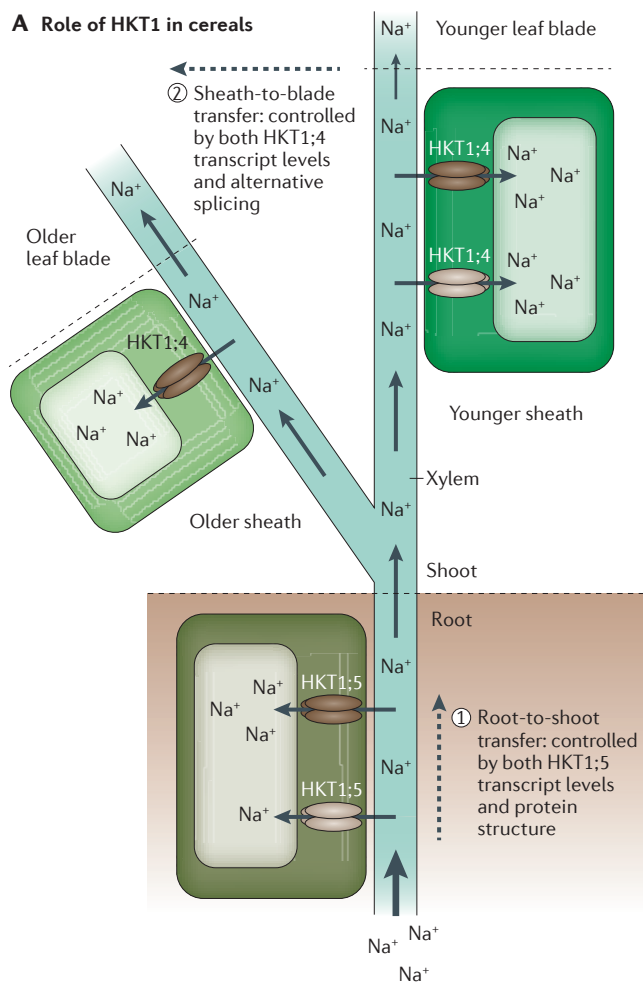
Guard cell

One of a pair of cells within the epidermal layer of some organs (primarily leaves) that are shaped to form a closable pore (stoma) that enables the exchange of gases between the plant and the atmosphere.

Stay-green phenotype

Phenotype of delayed senescence through the grain-filling period that is associated with delayed chlorophyll degradation.

A Role of HKT1 in cereals



B Nax1 and Nax2 durum wheat: genes from relatives

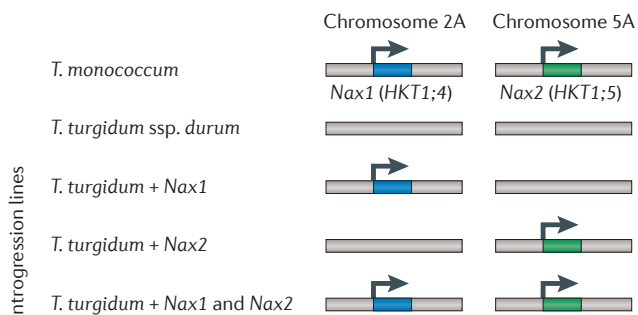
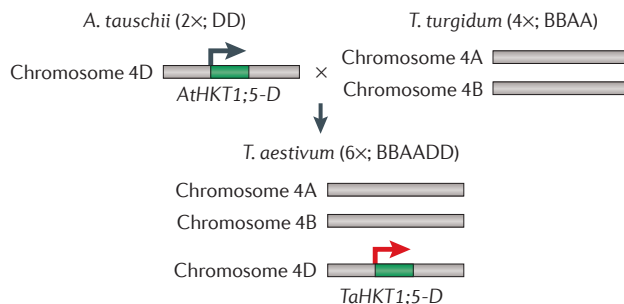
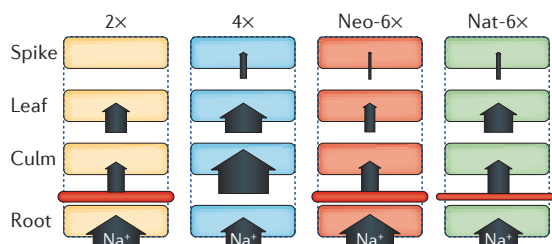


Figure 3 | HKT1 gene and functional variation associated with salt tolerance in the cereals wheat and rice. **A** | In cereals, high-affinity K^+ transporter1;5 (HKT1;5) facilitates Na^+ exclusion from root xylem vessels to reduce shoot accumulation⁷⁶, whereas HKT1;4 partitions Na^+ from the root xylem stream to leaf sheaths, reducing movement of the cytotoxic ion to photosynthetically active leaves¹⁵⁰. Salt tolerance of these cereals is linked to HKT1 locus integration and allelic differences in expression, activity and/or Na^+/K^+ selectivity. **B** | The Nax1 (HKT1;4) and Nax2 (HKT1;5) loci were introgressed from *Triticum monococcum* through a derivative durum wheat (*Triticum turgidum* ssp. *durum*)⁷⁷, resulting in improved salt tolerance. **C** | *Triticum aestivum* (bread wheat) contains the Kna1 (*TaHKT1;5D*; orthologous to Nax2) locus in the D genome (contributed by *Aegilops tauschii*), providing shoot Na^+ exclusion and salinity tolerance^{78,151}. Allohexaploidization⁶⁹ led to *TaHKT1;5D* neofunctionalization that was manifested by salt-responsive expression (as indicated by

Ca HKT bread wheat: allelic variation and neofunctionalization



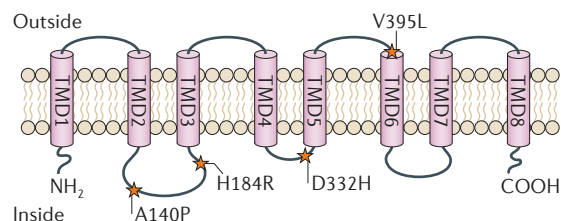
Cb



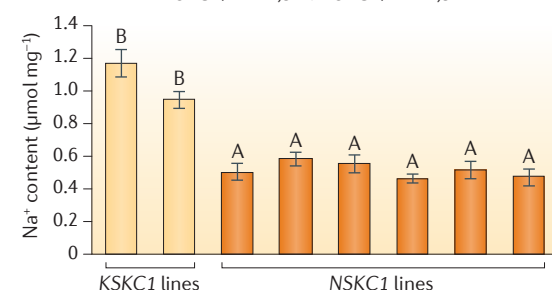
Da HKT rice: allelic variation



Db



Dc



the red arrow) (part **Ca**). AtHKT1;5 of the diploid (2x) *A. tauschii* limits Na^+ transport from roots to the culm (stalk), leaf and inflorescence (spike) tissues. Tetraploid (4x) *T. turgidum* transports more Na^+ to aerial tissues than both synthetic (Neo-6x) and naturally occurring (Nat-6x) hexaploid *T. aestivum* that carries *Kna1/TaHKT1;5D* (part **Cb**). **D** | Alleles of HKT1 of rice associated with sensitivity (Koshihikari; KSKC1 allele) and tolerance (Nona Bokra; NSKC1 allele) to salt are expressed in xylem parenchyma cells of shoots and roots⁸⁰ (part **Da**). Tolerance is associated with greater selective Na^+ transport by Nona Bokra HKT1;5 relative to Koshihikari HKT1;5 owing to four amino acid substitutions (orange stars) in loop domains (part **Db**) that result in lower Na^+ accumulation (part **Dc**). Columns with different letters indicate significant difference at $P < 0.01$ (least significant distance test); error bars are means \pm standard error. Part **B** adapted from REF. 150. Part **Cb** adapted from REF. 79, National Academy of Sciences. Parts **Db–Dc** adapted from REF. 80, Nature Publishing Group.

yield stability⁸⁴ (TABLE 2). Al³⁺-tolerating genotypes of cereals are characterized by enhanced Al³⁺-responsive transcriptional and post-transcriptional regulation that coordinates acclimation mechanisms⁸⁵. Primary Al³⁺ tolerance determinants are plasma membrane-localized transporters that efflux organic acids into the rhizosphere to reduce pH and to chelate the metal, making it unavailable for plant uptake. The first characterized organic acid transport gene shown to co-segregate with Al³⁺ toxicity avoidance encodes the wheat Al³⁺-activated malate transporter (TaALMT1)⁸⁶, which is apparently derived from several progenitor sources⁸⁷ and the allelic variation for which is associated with promoter strength owing to *cis*-element repeat number⁸⁸.

Subsequently, the multidrug and toxic compound extrusion (MATE) transporters that facilitate citrate export out of root tips were identified as Al³⁺-tolerance determinants in several grain crops. The barley HvAACT1 (MATE family) functions in cortical and epidermal cell citrate extrusion to the root xylem and is more highly induced by Al³⁺ in tolerant accessions⁸⁹. The tolerance allele selected during cultivation on acid soils is linked to a ~1-kb insertion in the 5' untranslated region that enhances Al³⁺-induced HvAACT1 expression in root tip cortical and epidermal cells. Allelic variation and CNV are associated with increased gene expression of MATEs and are causal for Al³⁺ tolerance conferred by specific haplotypes of the *Alt_{SB}* (encoding *SbMATE*) and the *ZmMATE1* loci of sorghum⁹⁰ and maize⁹¹, respectively (FIG. 4a). Transgenic expression of MATEs confers Al³⁺ tolerance (FIG. 4b), although translation to yield stability is not known. In rice, there is limited evidence of genetic variation for Al³⁺-induced organic acid efflux. Instead, tolerance involves regulation of Al³⁺ uptake and sequestration, which is associated with differential expression and transport properties of the plasma membrane Nramp Al³⁺ transporter 1 (NRAT1)⁹².

Boron (B) is an essential micronutrient that is required for several plant processes, including meristem development^{93,94}, that can be present at insufficient or toxic levels⁹⁵. Recent identification of B tolerance loci in barley and wheat uncovered genes with an adaptive advantage in B-rich environments that are absent from varieties grown in low-B soils. In barley, B toxicity tolerance is associated with CNV (four tandem copies) of *Bot1* (encoding a B efflux transporter), which is highly expressed in the tolerant landrace Sahara 3771 (REF. 96) (TABLE 2). A second barley B-tolerance locus encodes an aquaporin-type B transporter (HvNIP2;1) that facilitates B uptake⁹⁷. Levels of the transcript of this transporter are constitutively low in the roots of a B-tolerant genotype and are further reduced on B-rich soils, thereby limiting B influx. B tolerance in wheat is associated with a B transporter-like gene (*Bot-B5b*) of *A. tauschii* origin, which has high root mRNA expression in tolerant genotypes⁹⁸; conversely, intolerance is associated with gene loss or allelic variation that reduces *Bot-B5* expression, protein production or activity. Elite wheat cultivars with *Bot-B5* currently provide a significant yield advantage in the high-B soils of southern Australia⁹⁸.

Improved use of essential nutrients

Agricultural soil nutrient deficiencies continue to increase with intensive crop production⁹⁹. Model plant studies have characterized numerous ion transporters that can be manipulated to improve the uptake efficiency for nutrients such as Fe²⁺, N, P and Zn²⁺ (reviewed in REFS 73,100). As observed for drought tolerance, root architecture influences mineral nutrition, especially P uptake¹⁰⁰. Mechanisms of sensing and response to low inorganic phosphate (P_i) levels are coupled to strategies that enhance its capture from soils, including symbiosis with arbuscular mycorrhizae, increases in root-specific P_i transporters, exudation of organic acids and altered root architecture (enhanced lateral root and hair formation, altered growth angle and aerenchyma)¹⁰⁰. Key to a systemic low-P_i response is the phloem-based transport from the roots to the leaves of microRNAs that target the degradation of the *PHO2* mRNA, which encodes a ubiquitin E2 conjugase that negatively regulates the P_i starvation response¹⁰⁰.

The *Phosphorus uptake 1* (*Pup1*) QTL of rice, identified in the *aus* landrace Kasalath mainly grown in India and Bangladesh¹⁰¹, contains a chromosomal region that is absent in the *O. sativa* ssp. *japonica* reference genome¹⁰² carrying the *PHOSPHATE STARVATION TOLERANCE1* (*PSTOL1*) gene that is responsible for enhanced P_i acquisition¹³ (FIG. 4c). *PSTOL1* transcript levels are slightly upregulated under P_i deficiency in root and shoot tissues, and the encoded kinase aids early root development, which enhances overall root biomass under both P_i-replete and P_i-deficient conditions. Introgression of *Pup1* successfully increased low-P_i tolerance of the high-yielding *O. sativa* ssp. *japonica* variety Nipponbare under controlled conditions¹³, as well as in *O. sativa* ssp. *indica* varieties IR64 and IR74 in the field¹⁰² (FIG. 4d). *Pup1* introgression into varieties that are popular in regions with P_i-poor soils is underway (S. Heuer, Australian Centre for Plant Functional Genomics (ACPFPG); personal communication, 2015). In sorghum, allelic variation within four tandem-duplicated and one dispersed *OsPSTOL1* orthologue also contributes to grain production under P_i-limited conditions¹⁰³.

Reproduction and low-temperature tolerance

Temperate plants have evolved adaptive mechanisms and capacities to survive low temperatures^{104–106}. Endurance of near- and sub-freezing environments involves the active process of cold acclimation whereby exposure to non-lethal low temperatures enables plants to acquire cold hardiness for overwintering. The acclimations include changes in membrane composition, increased levels of hydrophilic peptides and compatible osmolytes, increased antioxidant capacity and anatomical changes that enhance mechanical strength^{105,107} (TABLE 1).

Freezing tolerance in temperate cereals is associated with transcription factors. In temperate cereals (monocots), including wheat and barley, two loci encoding transcription factors are associated with low-temperature tolerance and yield: the *FROST RESISTANCE1* (*FR1*) locus contains *VERNALIZATION1* (*VRN1*) and the *FR2*

Rhizosphere
Soil and associated microorganisms in the narrow region surrounding the root system.

locus contains *CBF* (also known as *DREB*) genes^{108–111} (FIG. 5; TABLE 2). Selective breeding for genotypes that can be planted in different seasons has uncovered natural genetic variation at these loci. Winter varieties sown in the autumn must cold acclimate to

overwinter, a process termed vernalization that requires the transcription of *CBF* genes and that is necessary for the transition from the vegetative state to the reproductive phase by the following spring. Spring varieties typically do not require vernalization and are also unable

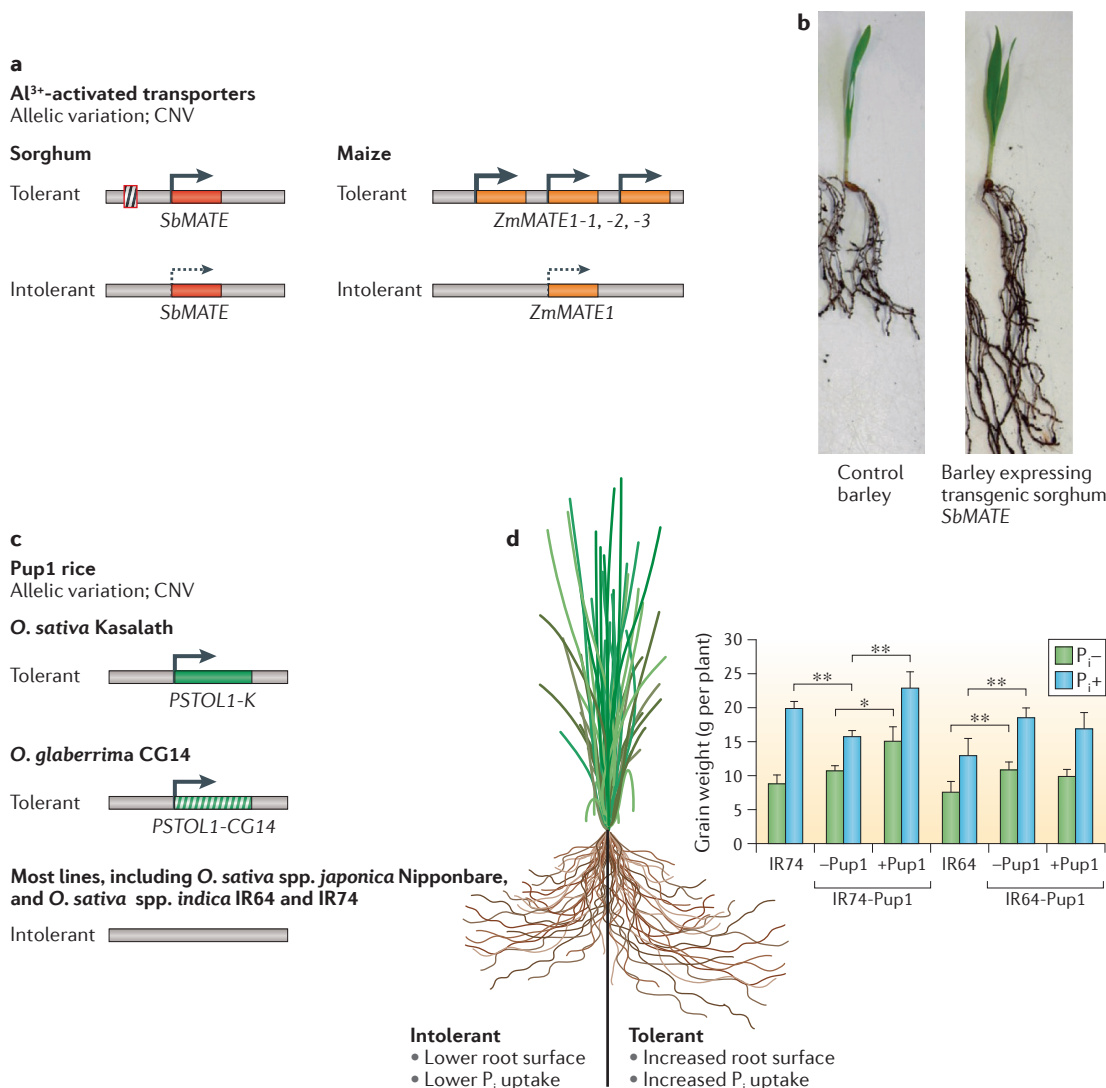


Figure 4 | Genetic basis for high Al³⁺ and low P_i tolerance mechanisms. **a** | The multidrug toxic compound extrusion (*MATE*) family is a key genetic determinant of Al³⁺ tolerance in many crop plants. Allelic variation in sorghum, wheat (not shown) and barley (not shown) is associated with regulatory sequences (striped rectangle) that allow upregulation in high-Al³⁺ soils^{84,85}. Tolerance to high Al³⁺ in maize is associated with copy number variation (CNV) of *MATE*s⁹¹. Extrusion of malate or citrate to the rhizosphere decreases the amount of soluble Al³⁺. **b** | Transgenic constitutively expressed sorghum *SbMATE* in sorghum (not shown) or barley increases Al³⁺ tolerance in hydroponic and soil-grown plants¹⁵². **c** | P_i deficiency in rice is alleviated by the kinase encoded by *PHOSPHATE STARVATION TOLERANCE1* (*PSTOL1*), which is absent in widely grown lines such as *Oryza sativa* ssp. *indica* varieties IR64 and IR74. A distinct functional allele is recognized in an African *Oryza glaberrima* accession (CG14)¹⁵³. **d** | Introgression of the *Phosphorus uptake 1* (*Pup1*) locus from the *aus*-type variety Kasalath, which contains *PSTOL1-K*, results in an expanded root system due to early root establishment¹⁰². Increased grain yield in low-P_i fields is conferred by *Pup1* introgressed into *O. sativa* ssp. *indica* varieties IR64 and IR74, as seen by comparison of near-isogenic sibling lines with the locus (+*Pup1*) and without the locus (–*Pup1*)^{13,102}. *0.05>P≥0.01, **0.01>P≥0.001. Part **b** is reproduced from Zhou, G. et al., Enhancing the aluminium tolerance of barley by expressing the citrate transporter genes *SbMATE* and *FRD3*, *J. Exp. Bot.*, 2014, **65**, 9, 2381–2390, by permission of Oxford University Press. Part **c** adapted from REF. 73, Nature Publishing Group. Part **d** republished with permission of American Society of Plant Biologists, from Developing rice with high yield under phosphorus deficiency: *Pup1* sequence to application, Chin, J. H. et al., *Plant Physiol.* **156**, 1202–1216 (2011); permission conveyed through Copyright Clearance Center, Inc.

Cold tolerance in wheat and barley CNV and allelic variation

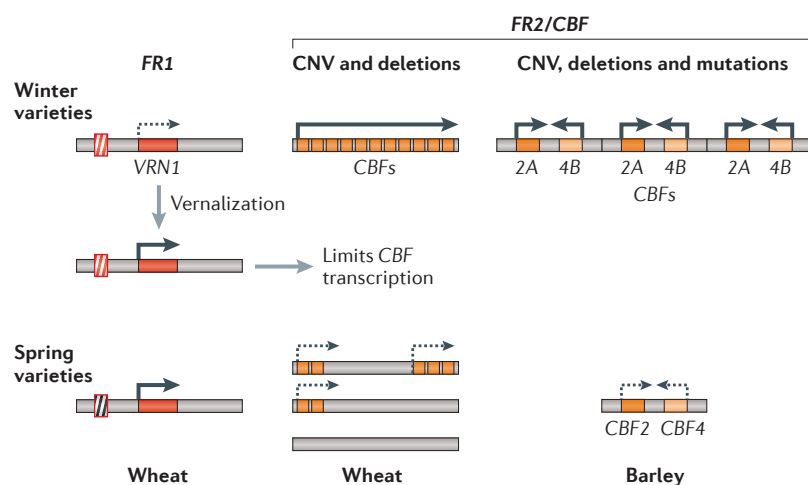


Figure 5 | Cold tolerance in temperate cereals is coordinated by *VRN1* at the *FR1* locus and by *CBF* genes at the *FR2* locus. *VERNALIZATION1* (*VRN1*) mRNA accumulation is elevated by vernalization (and short days) in winter genotypes but is constitutive in spring varieties. The thickness of the right-angled arrows represents transcriptional activity, and dashed arrows indicate low levels of inducible expression. The striped rectangles represent different regulatory sequences. In winter varieties, *VRN1* transcripts are limited until the vernalization requirement is met; during this period, high *CBF* expression facilitates cold acclimation for winter hardiness. After vernalization, elevation of *VRN1* expression promotes the reproductive transition and flowering, at which time *CBF* expression is repressed and growth is stimulated. Freezing tolerance of winter wheat and barley is associated with high *CBF* copy number and higher overall *CBF* expression. *CBF* gene deletion and mutation or lower levels of expression, as well as high *VRN1* transcription, are characteristic of spring varieties. Wheat *FR2* haplotypes with deletions encompassing 9 and 11 *CBFs* are susceptible to freezing¹¹⁹. *FR2* haplotypes of barley have few to >13 linked *CBFs*, with high copy number in winter-hardy genotypes. Copy number variation (CNV) includes segmental triplication of a *CBF2A* and *CBF4B* interval in the cultivar Dicktoo, contrasting with the single *CBF2* and *CBF4* segments in the spring varieties Morex and Tremois¹¹⁰.

to fully cold acclimate; therefore, they cannot tolerate freezing temperatures. *VRN1* encodes an APETALA-LIKE MADS box transcription factor that accumulates in response to cold and short days and that is a positive regulator of floral meristem identity and the developmental transition to flowering. *VRN1* transcript accumulation is limited in winter wheat and barley when first exposed to low temperatures, during which time freezing tolerance involving high *CBF* expression is acquired (FIG. 5). The prolonged exposure to cold and short day length during winter increases *VRN1* transcript accumulation, ultimately promoting the transition to flowering, which coincides with a reduction in *CBF* expression^{108–110}. In winter barley, this transition is correlated with histone H3 methylation changes in nucleosomes located in the promoter and the first intron of *VRN1* (REF. 112). Cold-sensitive (spring) barley genotypes are distinguished by constitutively expressed *VRN1* alleles that have promoter or first intron mutations that prevent negative regulation.

FR2/CBF genes regulate cold acclimation and freezing tolerance in annual or perennial monocots and dicots^{104,113}. In cereals, for example, *FR2* defines a region

containing numerous duplicated *CBF* genes, most of which are orthologous across the different *Triticeae* taxa, including some that are present as tandem repeats with highly conserved coding sequences^{110,114,115}. Freezing tolerance of winter relative to spring varieties of barley and wheat is associated with higher *CBF* gene copy number and sustained higher *CBF* transcript levels before vernalization^{109–111,114–119} (FIG. 5). Thus, cold acclimation is linked qualitatively to vernalization-activated *VRN1* alleles and quantitatively to the expression of *CBF* genes. The demonstration that transgenic overexpression of *CBF* genes is sufficient to increase cold hardiness under field conditions in woody species further illustrates the evolutionary importance of *CBF* regulation (see Supplementary information S1 (table)).

Cold tolerance of non-temperate cereals. Rice does not cold acclimate and is therefore hypersensitive to sub-freezing temperatures. However, its MYBS3 transcription factor is associated with low-temperature tolerance of seedlings¹²⁰ (see Supplementary information S1 (table)). The MYBS3 transcriptional network responds more slowly than the *CBF* pathway to low temperatures, regulating low-temperature tolerance genes such as those involved in the repression of the *CBF* pathway, which distinguishes it as an independent low-temperature responsive cascade¹²⁰. In addition, several QTLs^{121–123} have been identified that may be exploited to obtain low-temperature yield stability in rice.

Incorporation of heat stress tolerance

Heat stress causes substantial yield losses, as reproductive development is particularly vulnerable to heat. This includes the production and fertility of male gametes, pollen–pistil interactions and female fertility⁵. Following fertilization, high night temperatures reduce seed maturation, thereby affecting seed quality in many crops such as rice^{5,124,125}. Important acclimation mechanisms that maintain the key functions of respiration and photosynthesis include rapid alterations in gene expression, increases in heat shock protein (HSP) levels, modification of membranes, alterations in the cytoskeleton and management of oxidative stress. HSPs assist in protein folding and limit protein aggregation in multiple cellular compartments^{5,126}. Thermotolerance in several species is associated with higher levels of HSPs or their transcripts; however, improvement based on *HSP* expression invariably compromises growth under non-stress conditions^{5,127}.

A current approach to the challenge of high-temperature tolerance is ‘physiological’ or ‘developmental’ trait breeding through recurrent selection using crop germplasm from regions with hot growing seasons¹²⁸. In both cases, the targets are loci with high heritability that sustain yield at normal and elevated temperatures. The selection can be on combined heat and drought tolerance, as required by many crops. Recurrent selection has been successfully used to improve heat tolerance in wheat^{129,130} and potato¹³¹. In wheat, the stable introduction of chromosomes from its wild relative *Leymus racemosus* provided heat tolerance in hot and

arid fields¹³². The heat- and drought-tolerant rice variety N22 (*aus* ssp.) has provided QTLs associated with high levels of HSPs in anthers, spikelets and flag leaves associated with maintenance of yield under high night temperatures^{133,134}. Additional targeted developmental breeding in rice takes advantage of QTLs from *Oryza officinalis* that avoid heat-induced spikelet sterility by promoting dehiscence and fertilization in the cooler early mornings¹³⁵. In time, these approaches may yield loci and knowledge that can accelerate improvement in heat tolerance in combination with drought tolerance.

Climate-resilient crops: the path forward

Global climate change and environmental degradation are increasing the unpredictability and severity of abiotic stresses that have an adverse impact on the fundamental processes of growth and reproduction of plants, affecting global agriculture and food security (TABLE 1). It is imperative to improve crop yield stability in variable environments. This has been progressively accomplished through selective breeding using natural genetic variation that reflects the evolution of plants within diverse ecological niches, including extremes in water, temperature, nutrients and toxins. Expedient utilization of adaptation loci that improve yield under adverse growth conditions relative to existing crop germplasm depends on the ability to identify, map and move crucial loci into elite cultivars (TABLE 2). In many instances, knowledge of the molecular, cellular and physiological underpinnings of environmental adaptation obtained with model plants has proved essential in guiding the refinement from the genetic regions (for example, QTLs) to the functional determinants (such as genes and regulatory elements) within these regions. An important observation that has emerged is that biodiversity among causal stress adaptation loci often includes gene allelic variation, CNV and neofunctionalization.

As illustrated with examples for several environmental challenges, genetic diversity for stress tolerance that improves crop yield stability can be present in accessible gene pools (FIGS 1–5), including existing crop germplasm (that is, traditional landraces and varieties) or wild relatives^{136,137}. Genes that enable growth and reproduction in adverse environments can be absent from modern cultivars owing to their exclusion or loss during domestication or subsequent germplasm improvement, linkage to non-beneficial loci or drag on productivity in optimal environments. Useful loci and allelic variants often correspond to the downregulation or disruption of genes in susceptible crop genotypes. Continued and broader mining of germplasm is merited. For example, certain stress tolerances of maize can be enhanced by the identification, characterization and ultimate introgression of teosinte traits^{30,31,138}, with the same paradigm applicable to other cereals^{132,76,77}, soybean¹³⁹, tomato¹⁴⁰, potato¹³¹ and the cornucopia of secondary crops. In some cases, key adaptation loci can be introduced into a related species via segmental translocations¹⁴¹, as demonstrated with a rye–wheat translocation that enhances root biomass and provides drought resilience in wheat⁴⁵.

Effective stress adaptation determinants range in function from transcription regulators that modulate signalling and response networks to effectors such as membrane transport proteins that control some of the final steps in ion homeostasis. In some cases, determinants of yield stability under stress are conserved across species, as exemplified by *HKT* genes affording salinity tolerance in species as diverse as wheat^{76,77}, rice⁸⁰ and tomato⁸³. In other cases, determinants may not be equally effective throughout development. For example, anaerobic rice germination for robust early establishment is an important agronomic trait that does not seem to overlap with the submergence tolerance of older seedlings¹⁴².

Potential of adaptation or acclimation capacity may be associated with gene family expansion that often enhances functional activity or neofunctional specialization of paralogues. Determinant sequence polymorphisms can affect coding sequence function, changes in quantitative or qualitative transcription — including cell- and tissue-specific regulation — and/or inducer-responsive expression. This emphasizes the importance of multiple levels of fine-tuning of stress responses. More broadly, the characterization of halophytes such as *Eutrema salsugineum* has highlighted the importance of CNV and neofunctionalization in salinity tolerance¹⁴³, and these and other extremophiles may provide novel alleles of conserved determinant genes for crop improvement¹⁴⁴. Environmental stresses can be concurrent or sequential, and stress tolerances can be independent or interconnected, posing the additional challenge of proper stacking or pyramiding of traits that are synergistic or at least not antagonistic. A fortuitous example is the two-for-one tolerance that is provided by the *SbMATE* region of sorghum that confers tolerance to high Al³⁺ and low P_i, which is mobilized in soils by citrate extrusion¹⁴⁵.

Further identification, understanding and implementation of effective stress adaptation determinants in crops will require aggressive and targeted investments. A combination of approaches is required to accelerate the identification and characterization of specific loci that can be moved by molecular marker-assisted breeding into elite varieties, minimizing yield-adverse linkages. These include the utilization of diverse germplasm resources, high-throughput phenotyping platforms, and genome and exome sequencing of crops and their relatives. Furthermore, molecular genetic resources — including pipelines for genome-wide association studies, mutation detection, gene discovery and expression — and the associated ‘omic’ databases are needed. In particular, because tolerance of several abiotic stresses, including drought, flooding, nutrient deficiencies and toxicities, are dependent on the adaptation of roots, phenotyping platforms that visualize root system architecture should be a high priority¹⁴⁶. A deeper understanding and identification of the determinants that are involved in root sensing and root-to-shoot communication will also probably lead to improvements in stress tolerance.

With the rapid improvement of high-throughput field-based phenotyping platforms¹⁴⁷, the trait-to-gene-to-field approach is likely to become

increasingly expeditious. Although stress adaptation loci that have evolved in nature can be moved relatively quickly to the field via introgression into elite germplasm owing to the absence of regulatory issues, transgenes expressed constitutively or in an appropriate spatiotemporal manner are an important solution (see Supplementary information S1 (table)), especially when natural genetic diversity is not readily available. The targeted editing of genomes with transcription activator-like effector nuclease (TALEN)¹⁴⁸ and CRISPR–Cas9 (REF. 149) technologies is also likely to enable precision design of alleles that aid stress tolerance that can be readily adopted. Whether genes that limit yield loss under abiotic stress are natural loci transferred through

breeding, engineered genes transferred into plants, or *in situ* edited plant genes, the study of the underlying mechanisms, field performance and ecosystem effects is warranted.

The success stories highlighted here all include field assessment of yield, which is an essential step for proof-of-concept. Undoubtedly, to ensure global food security, anticipatory agronomic approaches and expansion of germplasm varieties, including greater adoption of ‘orphan’ crops that are particularly suited to certain climates, need to be pursued. To provide climate-resilient crops to farmers in the time frame needed, we must take advantage of both advanced technologies and evolutionary diversity in germplasm.

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Competing interests statement

The authors declare no competing interests.

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