SUMMARY
Salt stress limits the productivity of crops grown under saline conditions, leading to substantial losses of yield in saline soils and under brackish and saline irrigation. Salt tolerant crops could alleviate these losses while both increasing irrigation opportunities and reducing agricultural demands on dwindling freshwater resources. However, despite significant efforts, progress towards this goal has been limited, largely because of the genetic complexity of salt tolerance for agronomically important yield-related traits. Consequently, the focus is shifting to the study of traits that contribute to overall tolerance, thus breaking down salt tolerance into components that are more genetically tractable. Greater consideration of the plasticity of salt tolerance mechanisms throughout development and across environmental conditions furthers this dissection. The demand for more sophisticated and comprehensive methodologies is being met by parallel advances in high-throughput phenotyping and sequencing technologies that are enabling the multivariate characterisation of vast germplasm resources. Alongside steady improvements in statistical genetics models, forward genetics approaches for elucidating salt tolerance mechanisms are gaining momentum. Subsequent quantitative trait locus and gene validation has also become more accessible, most recently through advanced techniques in molecular biology and genomic analysis, facilitating the translation of findings to the field. Besides fuelling the improvement of established crop species, this progress also facilitates the domestication of naturally salt tolerant orphan crops. Taken together, these advances herald a promising era of discovery for research into the genetics of salt tolerance in plants.

INTRODUCTION
Salt stress is a major constraint on plant performance. Saline soils alone, which account for one-twentieth of the global total and one-fifth of irrigated lands, have been estimated to incur $27.3 billion in agricultural damages annually (Qadir et al., 2014). This is a clear motivation for alleviating yield losses in saline soils, but the potential benefits from unlocking saline water (about 98% of global water resources) to expand irrigated agriculture and relieve growing pressure on freshwater resources is likely to be far greater. As such, addressing the issue of salt stress in plants offers a compelling contribution towards meeting the 50% increase in demand for food and freshwater required to sustain 10 billion people by 2050 (Gleeson et al., 2012; UN Water and Energy, 2014; Mekonnen and Hoekstra, 2016; FAO, 2017). Despite optimistic projections at the global scale, food scarcity will continue to persist regionally, particularly in sub-Saharan Africa, the Middle East and South Asia where population growth is highest and agricultural outputs are most limited (FAO, 2017). In these most exigent regions, the attainable yield gap (i.e. the difference between observed yields for a given area and attainable yields under a similar climate) is substantial and constitutes a major challenge to present and future agricultural production. But given that 80% of the required increase in agricultural production by 2050 is expected to arise from yield increases (Alexandratos and Bruinsma, 2012) it also highlights an opportunity. For example, meeting attainable yields in rice, maize and wheat could increase production by 230%, 135% and 224%, respectively, in sub-Saharan Africa and 129%, 83% and 61% in South Asia (Mueller et al., 2012). An increase in irrigation alone could provide yield gains of over 100% for these major crops, yet access to freshwater poses a serious limitation (Mueller et al., 2012; Famiglietti, 2014; Rodell et al., 2018).
The development of crops with enhanced performance under saline conditions could enable the use of saline water sources, such as brackish water and partially desalinated or diluted seawater, for irrigation, and contribute to the sustainable intensification of agronomic systems under correct management.

Despite the potential of salt tolerant crops to significantly improve food production systems and the substantial efforts from the research community, relatively few salt tolerance genes or alleles have been identified and even fewer have led to real world applications. The genetic complexity of salt tolerance poses the greatest hurdle to progress. This review begins by revisiting how salt stress responses and salt tolerance are defined to provide a framework within which this complexity can be dissected to improve genetic resolution. We suggest that forward genetic approaches, genome-wide association studies (GWAS) in particular, can provide unique insight ‘from the plant’s perspective’ that can cut through this complexity and drive the discovery of new salt tolerance genes and alleles. We focus on the major challenges that have hindered such approaches in the past decade, alongside recent advances that are emerging as promising solutions and facilitators to uncover the genetic basis of salt tolerance.

**SALT STRESS: EFFECTS, RESPONSES AND PHENOTYPES IN PLANTS**

Salt stress can directly affect plant performance in several ways. High salinity causes a reduction in the osmotic and water potential of the growth medium, impeding water uptake. This, as well as the high levels of sodium ions themselves, can also affect nutrient absorption by disrupting native soil physico-chemistry and the function of uptake mechanisms. Sodium ions are also able to enter the root through constitutively expressed ion channels and transporters, eventually spreading throughout the plant via the vascular system. Over time, sodium ions can accumulate to such levels as to incur cytotoxic effects or to cause osmotic imbalances within tissues, cells and subcellular compartments. Accumulation, and therefore damage, tends to be greater in aerial tissues because sodium ions are delivered there as solutes in the transpiration stream – as water is lost through transpiration, sodium ions are deposited cumulatively. These combined effects lead to the impairment of vital biological processes.

Plants can respond in various ways to these challenges. The responses of plants to salt stress are systemic in scope, occurring in both roots and shoots, at the organ, tissue, cellular and subcellular scales. Common responses include processes such as stress sensing and signalling, regulation of ion homeostasis, metabolism, stomatal aperture, photosynthesis, cell division and expansion, as well as changes in plant morphology and architecture, phenology and resource allocation (reviewed by Munns and Tester, 2008; Hairmansis *et al.*, 2014). The responses employed, their nature, extent and the mechanisms that drive them can vary greatly between and within plant species. It is the interplay between the various direct effects of salt stress and the numerous potential plant responses that give rise to the wide diversity of phenotypic effects induced by salt stress that are observed across the plant kingdom.

In its broadest sense, salt tolerance is a measure of the maintenance of desirable plant performance traits under salt stress relative to control conditions. In practice, there...
is no single measure of salt tolerance, rather there are various indices by which maintenance of performance can be expressed, each having a slightly different focus (examples are presented in Table 1). For instance, the S/C index is the ratio of trait values under salt and control conditions, while the TOL index is the absolute difference between the two values. Generally, salt tolerance is measured using S/C index, due to its widespread use and broad relevance. There are also numerous plant performance traits that can be considered when evaluating salt tolerance. For application-oriented research, salt tolerance should ultimately be assessed on the basis of economically or societally relevant traits in crops. Yield is typically the most important, but biomass, fruit or grain quality and others may be valid, depending on the species and market demands. Other traits (e.g. transpiration, photosynthetic activity, metabolite profiles) should be considered, not as measures of salt tolerance in themselves but rather as potential factors contributing to salt tolerance in terms of these traits of real-world relevance.

Yield, for example, is a highly complex trait with many contributing factors. As a result of this complexity, it can be challenging to study the genetics of salt tolerance for yield in toto. Instead, it is more effective to study the genetics of the downstream traits that contribute to salt tolerance for yield. These contributing traits, their responses to salt stress and how they contribute to salt tolerance for yield can be dissected hierarchically (Figure 1). As such, salt tolerance for yield can arise from the maintenance of harvest index, i.e. the yield as fraction of overall biomass, which itself depends on maintained growth rates, in turn determined by traits such as transpiration, radiation and nutrient use efficiency (TUE, RUE, NUE, respectively), each of which are influenced by traits such as stomatal aperture and photosynthetic activity – and so on, down to the gene level. Each downstream component can contribute to salt tolerance for yield; thus constituting a salt tolerance mechanism. With a sufficiently comprehensive phenotyping methodology, insight into the eventual contribution of component traits to salt tolerance in terms of yield can be gained using correlation analyses. Although each of these components may remain complex themselves, this disambiguation helps to dissect salt tolerance for yield into constituent parts that are more genetically tractable than the whole.

The mechanisms that contribute to salt tolerance for yield, and subcomponents thereof, can vary between and within species, and also throughout development and across different environmental conditions. It is therefore important to account for factors such as genotype, salt stress concentration and distribution (e.g. temporal dynamics, topography), the developmental stage at which stress is imposed, weather conditions, cultivation practices, soil type and water availability (Greenway and Munns, 1980; Tal and Shannon, 1983; Mano and Takeda, 1997; Foolad, 1999; Yamaguchi and Blumwald, 2005;

### Table 1 Examples of stress tolerance indices

| Index                  | Description                                      | Source(s)                   |
|------------------------|--------------------------------------------------|------------------------------|
| Salt tolerance index   | $S/C = Y_S/Y_C$                                  | Munns (2002)                |
| Tolerance index        | $TOL = Y_C - Y_S$                                | Fox and Rosielle (1982), Fernandez (1992) |
| Mean productivity index| $MP = \frac{Y_C + Y_S}{2}$                       | Rosielle and Hamblin (1981) |
| Geometric mean productivity | $GMP = \sqrt{(Y_C \times Y_S)}$                | Fernandez (1992)            |
| Stress susceptibility index | $SSI = \frac{Y_C - Y_S}{Y_C + Y_S}$         | Fischer and Maurer (1978)   |
| Stress tolerance index | $STI = \frac{Y_C \times Y_S}{(Y_C)^2}$        | Fernandez (1992)            |
| Stress weighted performance | $SWP = \frac{Y_S}{\sqrt{Y_C}}$     | Saade et al. (2016)         |

$Y_C$ and $Y_S$ denote the value of the trait selected for assessing salt tolerance for a particular experimental unit, under control and stress conditions, respectively. $Y_C$ and $Y_S$ denote the population-wide yield under control and stress conditions, respectively.
Dissecting the genetics of salt tolerance

For application-oriented research, salt tolerance should be assessed according to the final trait of interest, most commonly yield—these are typically highly complex. Overall salt tolerance can be hierarchically dissected to identify downstream traits that are more genetically tractable. Traits that are seen to correlate with overall salt tolerance point towards processes that contribute to overall salt tolerance, i.e. salt tolerance mechanisms. A non-exhaustive list of salt tolerance-related traits and mechanisms is presented with a broad sense of their hierarchical organisation. This top-down approach can eventually lead to the identification of underlying genetic components. Importantly, salt tolerance can be calculated using various stress tolerance indices, each of which provides a different perspective and a distinct focus on different aspects of salt tolerance. Moreover, any measure of tolerance can be influenced by the conditions under which it is assessed (examples are illustrated in circles) and the causal mechanisms underlying each measure can be distinct. HI, harvest index; RMR, root-mass-ratio; RGR, relative growth rate; TUE, NUE, RUE, transpiration-, nutrient- and radiation-use efficiency, respectively; S/C, salt tolerance index (Munns, 2002); TOL, tolerance index (Fox and Roseli, 1982; Fernandez, 1992); MP, mean productivity index (Roseli and Hamblin, 1981); GMP, geometric mean productivity (Fernandez, 1992); SSI, susceptibility index (Fischer and Maurer, 1978); STI, stress tolerance index (Fernandez, 1992).

Figure 1. A plant scientist’s guide to dissecting salt tolerance.

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DEPLOYING EMERGING PHENOTYPING TECHNOLOGIES FOR COMPREHENSIVE SALT STRESS PHENOTYPING

Phenotyping the effects of salt stress in plants has traditionally been a relatively manual and laborious process. The focus has been on traits such as direct biomass measurements (e.g., shoot and root, fresh and dry), yield-related traits (e.g., total seed mass, 1000-grain weight, grain number), basic morphometric and architectural parameters (e.g., plant height, root length, number and length of side shoots, tillers, lateral roots etc.), visual scores of general plant health status (e.g., survival, senescence) and physiological measurements (e.g., ion contents, stomatal conductance, chlorophyll content), as well as laboratory-based analyses of biochemical traits (e.g., metabolite and hormone levels) and gene expression (Campbell et al., 2017; Klem et al., 2017; Tschiersch et al., 2017; Ubbens et al., 2018). These methods have served well, particularly for reverse genetics approaches that typically deal with relatively small numbers of genotypes and thus sample sizes. However, for practical reasons, they have proven difficult to apply to forward genetics experiments that generally deal with large numbers of genotypes and sample sizes. Certainly, forward genetics salt tolerance screens have been widely performed on a staggering number of genotypes, yet these have relied on a very small number of very simple traits such as survival at the young seedling stage. The recent emergence of high-throughput phenotyping (HTP) platforms is rapidly closing this gap, allowing the capture of large numbers of highly informative plant traits across vast experimental populations. Moreover, many of these new technologies are deployable in the field, offering much needed capabilities for studying key yield-related traits that are necessary for application-oriented research.

Indeed, the vast majority of research into salt tolerance has thus far been extensively performed using model plants such as Arabidopsis thaliana, primarily due to practical constraints and the resulting focus of resources (methodological, genomic and genetic) towards this convenient organism. Yet, while Arabidopsis has without a doubt been the single most important species for the advancement of plant science over the past decades, its contributions to application-oriented research into salt tolerance are somewhat constrained by its inherently low level of salt tolerance and lack of agronomically relevant yield-related traits (Moller and Tester, 2007).

The past decade has seen the emergence of numerous HTP systems, which overwhelmingly rely on frequent, non-invasive, automated imaging to phenotype tens, hundreds, even thousands of plants in a short time frame (Fur-bank and Tester, 2011; Ghanem et al., 2015; Junker et al., 2015). These systems facilitate the phenotyping of large numbers of genotypes in both controlled and field environments. The HTP platforms typically use red green blue (RGB), thermal infrared (TIR), chlorophyll fluorescence (ChIF) and, more recently, multispectral and hyperspectral imaging (see reviews by Humplik et al., 2015; Fahlgren et al., 2015). In RGB imaging, pixels are captured and subsequently analysed to inform on plant morphometric and colorimetric parameters, enabling the quantification of differences in growth rates or senescence, for example, under stress compared with control conditions (Rajendran et al., 2009; Al-Tamimi et al., 2016; Awlia et al., 2016). Thermal infrared imaging is used to measure canopy and plant heat signatures to provide indirect assessments of stomatal conductance and transpiration, which negatively correlate with leaf temperature (Merlot et al., 2002; Sirault et al., 2009). Measuring the photosynthetic performance of plants directly by measuring steady-state values upon light incidence or by observing the quenching kinetics of chlorophyll using ChIF imaging provides valuable insight into plant photosynthetic capacity and performance under stress (Baker, 2008; Jansen et al., 2009); (Campbell et al., 2015). Furthermore, multi- and hyperspectral imaging, which can capture narrow wavelength bands within and beyond the visible spectrum, show potential for the detection of stress responses (Fahlgren et al., 2015). Multi- and hyperspectral reflectance data are typically used to derive various vegetation indices that provide different estimations of the radiative properties of plant leaves or canopies. For example, the normalised difference vegetation index (NDVI) has been developed to assess plant health, while more complex spectral profiles afforded by hyperspectral imaging can be used to extrapolate the content of specific compounds, such as water, chlorophyll and even secondary metabolites like flavonoids and terpenoids (reviewed by Klem et al., 2017). The application of hyperspectral imaging for salt stress research is still in its infancy with only a few examples that focus on the estimation of vegetation indices as a proxy for crop stress (Naumann et al., 2009; Romer et al., 2012; Behmann et al., 2014; Sytar et al., 2017). Although hyperspectral imaging is more expensive than other imaging techniques and requires higher expertise for operation, image processing and data analysis and interpretation, it is expected to provide useful insights into the structural, biochemical and physiological traits related to salt stress responses. Recently, HTP methods have been widely employed to measure plants in the field by using these imaging tools with either ground-based or aerial imaging to monitor the responses of crops to stress (Baluja et al., 2012; Grieder et al., 2015). Unmanned aerial vehicles (UAVs), such as drones and fixed-wing aircraft, can measure plant traits at relatively high spatial and temporal resolutions. Moreover, they are becoming more affordable, accessible and common in crop research (reviewed by Araus and Cairns, 2014). However, data captured by UAVs require substantial computational power and highly trained personnel to
analyse them, which poses challenges to the deployment of UAV-based phenotyping platforms (Banan et al. 2018).

The major challenge of these imaging technologies, and HTP in general, is the massive number of data generated by these systems, which require sophisticated methods for data management, storage and analysis. To overcome this challenge, machine learning has become the leading method to facilitate data assimilation and trait identification for stress phenotyping (reviewed by Singh et al., 2016). New low-cost and open-source phenotyping technologies are continuously being developed, increasing their accessibility and usability for scientists and farmers alike (Tsafataris and Noutsos, 2009; Fahlgren et al., 2015). For example, the recently developed PlantCV is a low-cost HTP platform using low-cost Raspberry Pi microcomputers and cameras to acquire plant image data together with open source analysis software (Gehan et al., 2017; Tovar et al., 2018).

As HTP systems can be employed for measuring large numbers of complex traits, they have great potential to measure the physiological effects of salt stress. For example, monitoring plant growth daily upon salt stress allows the distinction between tolerant and sensitive genotypes (Rajendran et al., 2009). The HTP protocols have also been described in Arabidopsis using RGB and ChlF imaging, which can identify a set of robust traits, such as growth rate, greenness and non-photochemical quenching, for phenotyping the early responses to salt stress (Awlia et al., 2016). The use of HTP has allowed dissection of the genetic components of temporal salt stress responses in crops such as rice and barley (Hairmansis et al., 2014; Schilling et al., 2014; Campbell et al., 2015; Al-Tamimi et al., 2016). The availability of reference genomes and genotypic data-sets for several plant species has facilitated the association of genomic and phenotypic data using mapping methods to detect more quantitative trait loci (QTLs) that can significantly increase crop yield and stress tolerance in future breeding initiatives. The use of next-generation phenotyping with new imaging technologies and novel traits is also paving the way for modelling genotype-phenotype interactions to reflect processes affecting crop growth and yield (Cobb et al., 2013). Moreover, the advances in monitoring environmental conditions, with the use of sensor technology, should be exploited to correctly characterise salt tolerance in terms of its conditional factors, for example soil salinity, water availability or climate (Figure 1). As such, genotype × environment (G×E) effects can be considered in the association and phenotypic modelling of traits contributing to salt tolerance.

As these new HTP platforms become increasingly sophisticated and accessible, our ability to screen large numbers of genotypes for forward genetics approaches grows steadily. Yet, to fully seize the opportunity of these new capabilities there is a clear need for expansive germplasm resources to characterise and mine the genetic components of salt tolerance. Fortuitously, the phenotyping revolution has been accompanied by another technological breakthrough that is unlocking vast genetic resources: the explosion of DNA sequencing technologies.

**HARNESSING THE GENETIC DIVERSITY OF EXOTIC GERMPLASM**

Current agricultural systems overwhelmingly rely on a small number of highly productive crops. A mere 20 plant species are used to fulfil 90% of the world’s calorie requirements, with just three of these – rice, maize and wheat – supplying approximately 60% of the total (Massawe et al., 2016; Buchanan-Wollaston et al., 2017). Domestication of these food crops from wild species began more than 10 000 years ago, over the course of which the performance and genetic architecture of the original progenitors were radically transformed ( Tanksley and McCouch, 1997; McCouch, 2004). The selective breeding of a small number of wild varieties carrying beneficial traits, such as compact plant stature, non-brittle rachis and loss of germination inhibition, produced landraces with superior performance but also gradually eroded the genetic diversity in successive populations (Dempewolf et al., 2017). The contraction of crop genetic diversity has been further exacerbated by modern plant breeding whereby high-yielding elite cultivars are developed by crossing productive landraces while the wild ancestors with greater genetic variation but poor agronomic value are ignored (Gascuel et al., 2017). Such genetic bottlenecks have been confirmed experimentally (Haudry et al., 2007; Abbo et al., 2014). The inclination of farmers to switch from growing local varieties and landraces to genetically uniform and high-yielding varieties has led to the loss of approximately 75% of genetic diversity in crops (Buchanan-Wollaston et al., 2017). Importantly, as modern breeding tends to be carried out in optimal agricultural settings, the genetic components of disease and pest resistance, as well as tolerance to abiotic stresses such as salt stress, are often amongst the lost genetic fraction. As a result, attempts to identify genes conferring salt tolerance within commercial crop varieties have yielded limited results.

Several sources of genetic variation are available to complement the genetic paucity of modern elite cultivars. Genetic diversity generated artificially through mutagenesis has proven a valuable resource for various crop species, as variants can be directly produced in commercial germplasm (Caldwell et al., 2004; Mba, 2013; Nikam et al., 2015; Gulfishan et al., 2016; Çelik and Atak, 2017; Pando and Deza, 2017; Wang et al., 2017; Tu Anh et al., 2018). However, given that salt tolerance is most likely to arise from the concerted effects of numerous mechanisms, the potential to artificially create salt tolerant variants is surely limited. On the other hand, these are quite likely to emerge
through natural selection in plants that are exposed to harsh environmental conditions, as observed in landraces or wild relatives of crops (Mayes et al., 2012). Therefore, it is essential to study the available naturally diverse germplasm. Global genebanks have collected approximately 2 million distinct plant accessions, of which a high percentage are landraces and wild relatives of crops (Commission on Genetic Resources for Food and Agriculture, 2010). The prospect of using such germplasm repositories as sources of natural variation for tolerance to environmental stresses such as salt tolerance has been widely discussed (Mas-saw et al., 2015; Tsujimoto et al., 2015; Hanin et al., 2016; Ali et al., 2017; Buchanan-Wollaston et al., 2017; Dwivedi et al., 2017; Gascuel et al., 2017; Mahau’dhi et al., 2017; Zhang et al., 2017; Cheng, 2018). Assessments of genetic diversity have been performed for various crop germplasm collections, such as maize (Whitt et al., 2002; Liu et al., 2003; Patto et al., 2004; Laborda et al., 2005; Warburton et al., 2008; Prasanna, 2012; Zheng et al., 2013; Kuhn et al., 2014), wheat (Laido et al., 2013; Nielsen et al., 2014), rice (Cho et al., 2000; Temnykh et al., 2001; McCouch et al., 2002; Garris et al., 2005; Xu et al., 2012; Thomson et al., 2017), barley (Struss and Plieske, 1998; Parzies et al., 2000; Fernandez et al., 2002; Moragues et al., 2010) and tomato (Albrecht et al., 2010; Bauchet and Causse, 2012; Affitos et al., 2014; Pailles et al., 2017). Furthermore, various studies using forward genetics approaches have already demonstrated the value of utilising diverse germplasm in practice by detecting loci associated with various measures of salt tolerance in established crops, for example in maize, rice, wheat, barley, soybean, rapeseed and alfalfa (Cu et al., 2015; Al-Tamimi et al., 2016; Liu and Yu, 2017; Wan et al., 2017; Zeng et al., 2017; Oyiga et al., 2018).

Overall, investigations into natural variation for salt tolerance remain scant considering the potential and the available germplasm resources. This is true for major crop species, but even more so for underutilised or orphan crops. Despite showing great potential in terms of salt tolerance, these valuable genetic resources have remained comparatively untapped. This is mainly due to the relative intractability of these germplasms. For instance, it can be challenging to rigorously phenotype highly diverse and undomesticated germplasm panels. Limited genetic and genomic resources for these germplasms as well as difficulties in effectively transferring beneficial alleles into elite varieties have also proved to be significant obstacles (Mayes et al., 2012; Wang et al., 2017). Nevertheless, the recent technological breakthroughs in the fields of genotyping, DNA sequencing and molecular-assisted breeding are increasingly facilitating the use of these genetic resources for crop improvement. For example, quinoa (Chenopodium quinoa), an undomesticated pseudo-cereal that has gained popularity in developed countries due to its nutritional potential (Cheng, 2018), displays substantial genetic and phenotypic diversity (Figure 2), including accessions with high tolerance to salt stress (Jacobsen et al., 2003). The recent publication of two high-quality quinoa genomes, alongside genomic data for a growing number of accessions and close relatives, provides great promise for detecting the genetic components underlying this species’ elevated salt tolerance (Jarvis et al., 2017; Zou et al., 2017). Interestingly, attempts are currently being made to domesticate these already salt tolerant orphan crops ahead of transferring this tolerance into major crops.

The accelerated development of DNA sequencing and genome assembly capabilities over the past decade is increasingly facilitating the genomic characterisation of new and uncharacterised germplasm — potential repositories of salt tolerance alleles and genes. Reference genome sequences are now available for over 300 plant species (as of March 2018; http://www.plabipd.de/), with chromosome-level assemblies for several major crops including rice, maize and wheat as well as less established species such as quinoa (Schnable, 2012; Mayer et al., 2014; Jackson, 2016; Jarvis et al., 2017). While the ever-decreasing cost of short-read sequencing platforms has driven much of this progress, it is the emerging long-read, real-time, linked-read and single-molecule sequencing techniques that are allowing researchers to overcome the intrinsic challenges of plant genomes, namely their large size, frequently complex ploidy levels and high repeat content (Bevan et al., 2017). The resulting high-quality reference sequences provide the basis for genome analyses to identify structural variants responsible for species-specific traits (e.g. the genes involved in fleshy fruit evolution in Solanum lycopersicum, or triterpenoid saponin biosynthesis in C. quinoa) (Sato et al., 2012; Jarvis et al., 2017). Such analyses have also provided insight into the potential genomic basis of drought and salt tolerance in the wild tomatoes Solanum pennellii and Solanum pimpinelli-folium, respectively (Bolger et al., 2014)(Razali et al., 2018).

The greatest value of these genomic resources lies in their ability to empower the capture and characterisation of genetic variation. Indeed, high-quality genomes beget high-quality genotyping, from well-established genotyping chips to more recent genotyping-by-sequencing methods, including various reduced representation sequencing and whole-genome re-sequencing approaches (Bevan et al., 2017; Dwivedi et al., 2017; Scheben et al., 2017). Using these approaches, vast numbers of accessions can now be genotyped relatively quickly and cheaply, with the potential to discover millions of single nucleotide polymorphisms (SNPs). Extensive genotypic resources are thus available for all sequenced major crop species, a number of wild relatives and increasingly for the aforementioned orphan crops, including diversity panels and mapping populations (e.g. bi-parental, nested association) (reviewed by (Rasheed et al., 2018)). These growing resources will
Figure 2. The phenotypic diversity of Chenopodium quinoa germplasm. The genetic diversity of quinoa germplasm can be clearly seen through the wide variety of panicle shapes, sizes and colours displayed in these field-grown specimens (30 distinct accessions grown in the same season and field) (photo credits: Gabriele Fiene).
facilitate continued and improved investigations into the genetic basis of salt tolerance.

Beyond improvements in cost and throughput, increases in coverage, accuracy and the ability to discriminate haplotypes between homologous chromosomes offer growing insight into structural variations such as copy number variants (CNVs), presence–absence variants and chromosomal rearrangements (insertions, deletions, inversions, translocations) that are typically not captured by SNP-based approaches. Our greater understanding of these structural variants, in particular CNVs that are widespread in plant genomes where they have been shown to contribute significantly to phenotypic variation, will provide much-needed progress for the improvement of crop salt tolerance. Furthermore, the increased potential for de novo genome assembly for large numbers of accessions from a given species or closely related species facilitates the compilation of pangenomes, which summarise the structural variation observed across a large number of genotypes, providing a more comprehensive insight into structural variation (Golicz et al., 2016). Pangenomes have been compiled for many major crop species such as maize, rice, hexaploid wheat, soybean and Brassica oleracea, but have yet to be fully explored in terms of connecting genetic and phenotypic variation, particularly with regard to salt tolerance (Hirsch et al., 2014; Li et al., 2014; Golicz et al., 2016; Montenegro et al., 2017; Zhao et al., 2018).

The increasing availability of reference genomes and genotypic resources for exotic plant species, alongside the continuous development of tools to rigorously phenotype various components of salt tolerance, will enable the scrutiny of these exotic germplasm resources for salt tolerance, the underlying mechanisms and their genetic basis. Such applications will continue to rely heavily on existing and emerging statistical approaches that detect associations between genetic markers and phenotypic data through GWAS.

GOING BEYOND CONVENTIONAL MAPPING

In forward genetic studies, the population size, phenotyping accuracy and linkage/association populations need to be carefully considered (Zhao et al., 2007). Striking a balance between the number of screened plant accessions, duration and temporal resolution time, traits to be measured and phenotyping precision are essential parameters to consider when planning an experimental setup that can reliably detect plausible loci with high statistical power. For marker-trait association mapping, GWAS can provide insight into complex trait architecture, highlighting potential QTLs affecting traits that contribute to salt tolerance depending on the model used (Korte and Farlow, 2013). The basic and original approach in GWAS is to evaluate the association between each genotyped marker and a phenotype of interest that has been scored across a large number of individuals. The widely used conventional mixed linear model (MLM) association using the efficient mixed-model association (EMMA) algorithm creates a linear mixed model that corrects for population structure using a marker-based kinship matrix (Kang et al., 2008). In this section, available GWAS methods and models are summarised, presenting the advantages and disadvantages of each, for easy reference for plant scientists (Table 2).

There are a large number of software packages that facilitate essential GWAS analyses for estimating genotype-phenotype associations, but very few published comparisons of the available methods by which an informed selection can be made. The most commonly used packages for applications in plant science are TASSEL, GAPit and PLINK, with a growing number of studies being carried out using the R statistical computing software, which allows more flexibility in the development of complex genetic association models. However, no available software or R package has been able to provide the ideal balance between advanced statistical methods, time-efficient simulations, accessibility and user-friendliness. With the major developments in phenotypic technology and exploration in analytic approaches, GWAS has become an even more effective and efficient technique for studying the genetics underlying trait variation. Many improved statistical methods to increase statistical power, reduce false-negative rates and incorporate biological context in GWAS results have recently been proposed in the field of plant genetics. Moreover, advanced forward genetics experimental setups, such as those that measure many traits over time for a large number of plants, are computationally demanding, adding to the requirement for more efficient methods for analysing high-throughput phenotyping data. For this purpose, longitudinal genome-wide association models have been developed to elucidate the genetic basis of the dynamic responses of rice growth to salt stress (Campbell et al., 2015). The model used accounts for both genetic relationships between genotypes due to the rice subpopulation structure and the inherent non-independent nature of daily observations.

Mixed models and Bayesian approaches are being explored to take into account environmental and treatment effects, while other models account for the effects of genetic background, interactions between multiple phenotypes and confounding phenotypes. Studies have used phenotypic covariates to address relationships among traits. An alternative GWAS interaction model was developed which integrates control and saline conditions. This interaction model incorporates the ‘main effects’ of the marker (SNP effect) and treatment (control or salt) as well as the marker-by-treatment interaction (the SNP effect in response to the treatment). The use of this interaction model enabled the identification of significant loci.
Table 2 Comparison of available methods and models for genome-wide association studies

| Method              | Description                                      | Tools                                                                 | Literature                        | Benefits                                                                                           | Limitations                                                                                           |
|---------------------|--------------------------------------------------|----------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Single locus method |                                                   |                                                                      |                                   |                                                                                                    |                                                                                                       |
| Exact methods       |                                                   |                                                                      |                                   |                                                                                                    |                                                                                                       |
| EMMA                | Efficient mixed model association                | TASSEL (http://www.maizegenetics.net/tassel), EMMA R package         | Kang et al. (2008)                | Polygenic variance is re-estimated with each marker analysed                                       | Computationally intensive, only allows a single effect (samples or taxa) to be fit as a random effect. All other effects are treated as fixed |
| GEMMA               | Genome-wide efficient mixed model association    | GEMMA software (http://stephenslab.uchicago.edu/software.html)        | Zhou and Stephens (2012)          |                                                                                                    |                                                                                                       |
| FaST-LMM            | Factored spectrally transformed linear mixed models | FaST-LMM (python)                                                    | Lippert et al. (2011)             |                                                                                                    |                                                                                                       |
| Approximate methods |                                                   |                                                                      |                                   |                                                                                                    |                                                                                                       |
| EMMAX               | Efficient mixed-model association expedited      | TASSEL                                                              | Kang et al. (2010)                | Scales linearly with cohort size in both run time and memory use, substantially increase speed     | Do not involve re-estimating polygenic variance, less accuracy, systematic and appreciable underestimation of the most significant P-values |
| CMLM                | Compressed mixed linear model                    | TASSEL, GAPIT (http://zlab.net/gapit)                               | Zhang et al. (2010)               |                                                                                                    |                                                                                                       |
| P3D                 | Population parameters previously determined      | TASSEL                                                              | Zhang et al. (2010)               |                                                                                                    |                                                                                                       |
| Multi-locus methods |                                                   |                                                                      |                                   |                                                                                                    |                                                                                                       |
| MLMM                | Multi-locus mixed model                          | Python(https://github.com/bvlilja/mixmogam/), R                     | Segura et al. (2012)              | Identify evidence for allelic heterogeneity as well as interactions, unbiased analysis for interactions within the selected set of single nucleotide polymorphisms (SNPs), to some extent handle the confounding usually attributed to population structure, increased power, yielding reliable results for large datasets | Forward-backward inclusion of SNPs limits exploration of the huge model space                             |
| LMM-Lasso           | Least absolute shrinkage and selection operator   | Python(https://github.com/borgwardtlab/lmm-lasso)                    | Rakitsch et al. (2013)            |                                                                                                    | Computationally demanding                                                                                 |
| BSLMM               | Bayesian sparse linear mixed model               | GEMMA software (http://stephenslab.uchicago.edu/software.html)       | Zhou et al. (2013)                |                                                                                                    |                                                                                                       |
| Multi-trait methods |                                                   |                                                                      |                                   |                                                                                                    |                                                                                                       |
| MTMM                | Multi-trait mixed model                          | R software(https://github.com/gregor-mendel-institute/mtmm)          | Korte et al. (2012)               | Considers both the within-trait and between-trait variance components                              | Difficult to implement in natural population-based mapping owing to computational complexity; cannot control for population structure |
| Interaction model   | Marker by treatment interaction model             | Asreml R (http://datadryad.org, doi:10.5061/dryad.3116j)             | Al-Tamimi et al. (2016)           | Incorporates ‘main effects’ of the marker (SNP effect) and treatment as well as the                | Cannot handle missing data                                                                                |
specifically associated with salt stress (Al-Tamimi et al., 2016). Another approach can be used to manage correlated phenotypes through deriving a fully parameterised multi-trait mixed model (MTMM) that considers both the within-trait and between-trait variance components simultaneously for multiple traits. This GWAS approach was first used for an Arabidopsis dataset of flowering measurements in two different locations, identifying loci whose effects are specifically determined by the environment (Korte et al., 2012). A study in rice controlled for the confounding effects of flowering time on panicle architecture, by eliminating SNPs associated with the former, making it easier to investigate loci associated uniquely with panicle phenotypes and their effects on yield (Crowell et al., 2016). Such approaches can greatly improve the efficacy of GWAS approaches and their use is strongly encouraged for elucidating complex phenotypes such as salinity tolerance.

The conventional EMMA method is based on single-locus tests (Kang et al., 2008), but as traits can be controlled by many loci with broader effects, as is the case for salt tolerance, this model does not produce a good estimate of marker effects. Numerous multi-locus methodologies, which assume that several loci contribute to the phenotype of interest have been suggested, including penalised regressions (Hoggart et al., 2008; Yi and Xu, 2008; Wang et al., 2011; Waldmann et al., 2014) and even the elastic net (Waldmann et al., 2014). Another multi-locus mixed model method involves the EMMA expedited (EMMAX) algorithm, which approximates the genetic and residual variance components and uses the same variance for all SNPs, re-evaluating the genetic and residual variances at each step of the algorithm to avoid repetitive estimation of the variance component for each SNP (Segura et al., 2012). An example of a multi-locus MLM used for salt tolerance traits in rice showed 20 quantitative trait nucleotides (QTNs) associated with 11 traits, including six QTNs affecting salt tolerance at the germination stage and 14 QTNs at the seedling stage (Naveed et al., 2018). Bayesian approaches have also extended to genetic association studies because of the practical and theoretical advantages shown in recent papers (Marchini et al., 2007; Servin and Stephens, 2007; Wakefield, 2007; Verzilli et al., 2008; Fridley, 2009). A number of software packages, for example SNPTEST (Marchini et al., 2007) and BIMBAM (Servin and Stephens, 2007) enable simple genome-wide Bayesian analyses to be done easily and quickly on any standard desktop computer. Additionally, Wang et al. (2012) proposed new GWAS approaches called GWAS by single-step genomic best linear unbiased prediction (ssGBLUP) and single-step GWAS (ssGWAS). The ssGWAS method provides the most comprehensive information for genomic evaluation. The ssGWAS model allows all the available data, including genetic markers, phenotype records and
pedigree information, to be examined simultaneously in one step. Many studies have recently validated this method and effectively implemented ssGWAS in pigs (Howard et al., 2015; Wu et al., 2018) and other species (Silva et al., 2017), achieving a greater power and more precise estimates than other models. The ssGWAS method is currently being tested in plant species by exploring the use of raw data (individual replicates) as opposed to standard approaches that use the means (across replicates), which leads to error inflation for the association analysis (unpublished data). Thus, genetic markers, the replicate measurements of each trait, spatial and design factors, and marker–treatment interaction can be simultaneously considered in a single model. Results from this approach are expected to increase in statistical power as each individual plant is considered as the input data instead of using the means of each genotype. This approach could be beneficial for complex quantitative salt tolerance traits that have various components that can be dissected effectively by a ssGWAS model, as we are able to detect significant loci specifically associated with salt stress due to the incorporation of the interaction model in the ssGWAS.

These rapidly evolving genome-wide association models in crops are promising and highly useful tools for allele and marker discovery.

**FUTURE DIRECTIONS**

To provide new insights into genotype-phenotype relations in terms of salt stress responses and tolerance in plants, the focus lies in the rapid advances of DNA sequencing technologies and HTP. This exciting era of omics tools promises to deliver powerful genetic and phenotypic resources that will enable a new generation of salt tolerant crops. The knowledge generated by these resources is expected to improve: (i) longitudinal GWAS combining HTP and new association methods (similar to what has been used in other research areas; Ning et al., 2017; Vujkovic et al., 2016); (ii) the genetic power to pinpoint the causal gene underlying a specific locus, for example, in Arabidopsis, the availability of 10 million SNPs has allowed the mapping of significant SNPs resulting from GWAS and specifically marking them by saturating the genome browser, thus uncovering the causal genes; and (iii) breeding programmes through identification of accessions carrying causal SNPs as donors for crossing schemes, and the use of causal SNPs to drive marker-assisted selection. Moreover, speed breeding methods (Watson et al., 2018), which can achieve up to six generations per year instead of the typical two to three is expected to contribute to the fast delivery of salt tolerant crops.

Rapid advances in breeding strategies, together with the availability of new genomic tools have paved the way for the use of genomic selection (Crossa et al., 2017). Genomic selection uses a training population of individuals that have been phenotyped for the trait of interest and genotyped with genome-wide markers that predict the breeding value of these individuals to enable the selection of superior lines and predict the performance of a genotype (Jannink et al., 2010). For example, different genomic models were tested to facilitate the selection of superior genotypes and candidate genes for breeding drought tolerance in subtropical maize germplasm (Shikha et al., 2017). The conjunction of genomic selection with the information provided by population structure, GWAS and the known genetic architecture of specific traits has successfully led to increased breeding efficiency in crops such as rice (Spindel et al., 2015). To our knowledge, genomic selection has not yet been used for breeding for salt tolerance in any plant species. However, we anticipate that the decreasing costs of genotyping and improved genomic models to predict breeding values will greatly contribute to accelerating the delivery of superior breeding lines with salt tolerance.

Moreover, the adoption of genome editing tools will enable the validation of causal gene function. For example, CRISPR-Cas9 is expected to elucidate causal relationships between candidate genes identified by GWAS and phenotypic traits for the implementation of targeted molecular-assisted breeding programmes (Yin et al., 2017). However, genome editing will not only assist the functional validation of loci of interest but will also enable the simultaneous modification of multiple genetic loci in elite crops, thus advancing breeding programmes and the translation of findings to the field.

Genetic diversity is a key asset for improving yield stability and stress tolerance. To improve agriculture, researchers use germplasm of crops and their wild relatives adapted to different environments as genetic resources ( Tanksley and McCouch, 1997; Mickelbart et al., 2015). The use of wild relatives should not be confined to their use for increasing the genetic diversity of elite crops. One may consider the hypothesis of directly domesticating minor and orphan crop species with higher salt tolerance. Quinoa, for example, has a high potential for development as it exhibits a high tolerance to several abiotic stresses including salt tolerance ( Jacobsen et al., 2003; Bazile et al., 2016; Jarvis et al., 2017), and thus breeding for important agronomic traits, such as shorter plants with fewer branches and more compact seed heads, will encourage commercial production in sub-optimal environments where salinity is a major obstacle.

Here we have emphasised the importance of field HTP using UAVs. Quantitative trait locus mapping using traits collected by UAVs has enabled the identification of different alleles contributing to the rapid selection of high-yielding varieties in rice (Tanger et al., 2017). We anticipate that machine learning algorithms will help leverage the outputs from emerging imaging technologies such as
hyperspectral and thermal imaging to drive advances in the identification of genomic regions controlling the components of salt tolerance in important crop species. Finally, it is worth highlighting that all the strategies discussed in this review can certainly be expanded beyond research into salt tolerance. For example, harnessing new genetic resources such as understudied crops or taking advantage of new imaging techniques using HTP along with new statistical association models will accelerate the discovery of new mechanisms of stress tolerance for any given stress. By establishing the connection between genotype and phenotype, we can advance the selection of high-yield stress tolerant crops and improve agricultural production to address the growing pressures on food security.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

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