



# CROP BIOTECHNOLOGY FOR IMPROVING DROUGHT TOLERANCE: TARGETS, APPROACHES, AND OUTCOMES

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**Abstract:** Human population growth and climate change threaten our food and water security. The increasing frequency of extreme drought events will cause major crop yield losses. To mitigate this threat to global food security, we need to rapidly select and/or develop new 'climate-ready' crop varieties that can withstand and flourish under water deficit, enabling the sustained and sustainable production of higher yields to support human life on Earth. In this article, we identify the current targets for crop plant improvement under drought, working from the ground up, with modifications in rooting, shoot, stomatal, and photosynthetic systems, and finally nutrient transport and sink strength. We argue that by using a holistic approach to crop development, prudently incorporating the natural variation available in crop wild relatives and cultivars with cutting-edge tools, such as molecular breeding and transgenics, we may be able to produce high-yielding crops under a range of conditions to meet our needs in a changing world.

**Keywords:** water deficit, crops, drought, roots, stomata, photosynthesis, translocation, source–sink, carbon metabolism, plant biotechnology

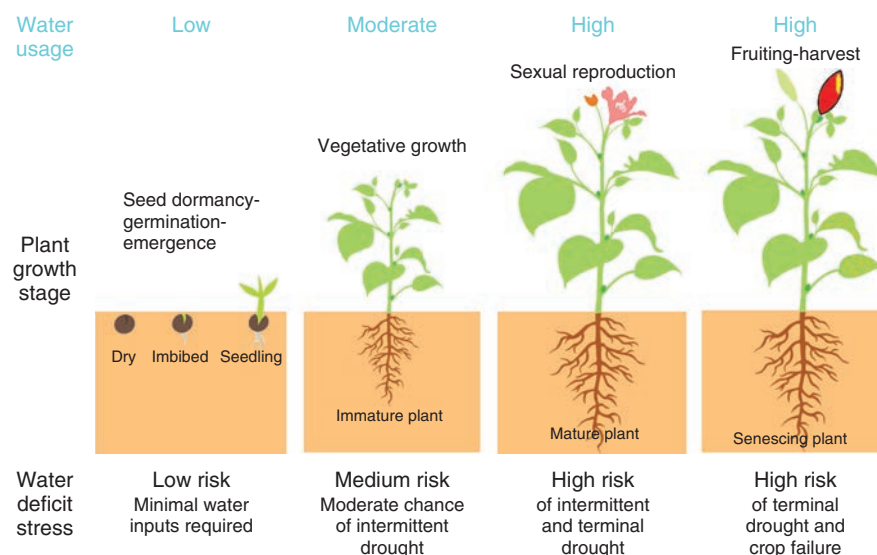
## 1 Introduction

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Water availability is the most important environmental factor limiting crop production (Boyer, 1982), not just in arid environments but in temperate zones as well. Global agriculture uses about 70% of freshwater resources and the unstoppable rise in the human population presents a conflict between water demands for food production and for direct human use (WWAP, 2015). Anthropogenic climate change, resulting from increasing fossil fuel-derived atmospheric CO<sub>2</sub> concentrations, is causing global temperature and atmospheric vapour pressure deficit (VPD) increases. These complex changes are disrupting previously stable climate and weather patterns and altering the timing, distribution, and degree of precipitation across the planet (Stocker et al., 2013).

Modern crop breeding for drought tolerance has had decades of success, allowing new varieties, cultivars, and elite germplasm to produce better yields under water deficit. Modern plant biotechnology, however, over the past thirty years or more has focused mainly on disease and insect resistance, as well as weed management. Very recently, Argentinian scientists from Verdeca have developed and released a drought-resistant transgenic soybean HB4 (Waltz, 2015). Before this, there was only one commercially available transgenic crop available on the market developed for drought resistance: Monsanto's DroughtGard maize hybrid. Syngenta's Agrisure Artesian and DuPont's Aquamax, drought-resistant corn hybrids, were bred using target gene and marker-assisted breeding, respectively. While the need for drought-resistant crops is increasing with the increasing incidence of climate change-related water deficits, the development of drought-resistance through biotechnology is in its infancy and often remains the domain of model plant or crop plant research under controlled environment or greenhouse conditions.

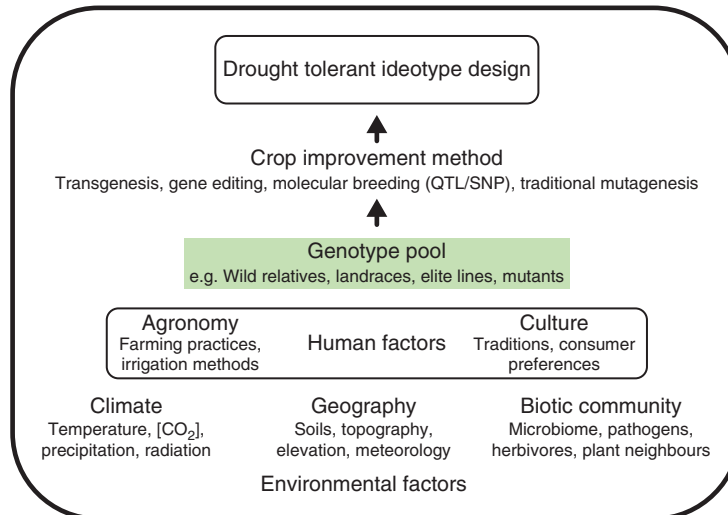
Two major hurdles in the development of drought-resistance biotechnologies are the facts that no one 'drought' scenario is the same and that responses are highly complex. Whereas often a single gene can be used successfully to confer herbicide tolerance or to alter fruit ripening, drought responses are rarely governed in this manner. Instead, the target traits required are themselves highly complex and multigenic. Drought stress is notoriously difficult to simulate in laboratory, greenhouse, and controlled environment experiments. Field experiments too have major drawbacks and, like the lab, the imposed nature of the water deficits are very difficult to replicate, in terms of degree of stress (change in substrate and tissue water potentials), rate of change of the stress (time of onset), and maintenance of the stress (duration) (Figure 1). In the natural environment and in agriculture, drought stress takes many forms, the severity and nature of each depending on the interaction between the genetic background (particular plant species/ecotype/landrace/variety, etc.) (Figure 2), its growth stage



**Figure 1** Plant development stages and susceptibility to different forms of water deficit. Crop plant water usage varies across its life cycle, as does the risk of water deficit. A dry seed is generally highly resistant to drought, and a germinating seed and seedlings (although highly sensitive to water deficit stress) are usually not affected by drought due to agronomic practices such as timing of sowing, burial in the soil profile, and low water requirements. The young crop, undergoing vegetative growth, is usually at low risk of drought stress, because the root system has developed sufficiently to support the relatively small above-ground biomass which still has low water demands. Mild and intermittent drought at this time can, however, reduce overall yields. The mature plant, during inflorescence production, fertilisation, and fruit-set is often at the highest risk of drought-induced yield losses as water deficits can directly induce flower, embryo, and fruit abortion. Furthermore, rainfed agriculture often relies on continued seasonal rainfall to ensure sufficient production prior to harvest.

(Figure 1), and the interaction between the individual plants' biotic and abiotic environment.

Confounding biotic variables that impact on drought stress response include the presence or absence of plant symbionts including rhizobial bacteria and mycorrhizal fungi, the role of herbivores and pathogens, and the role of neighbouring plants. Confounding abiotic variables include substrate composition (edaphic factors), aspect and elevation (geographic factors), air temperature, wind, atmospheric carbon dioxide concentration, season, etc. (Figure 2). 'Drought' *per se* is, therefore, an almost meaningless concept, but one that has been discussed and modelled across countless academic publications, all with the shared factor of some level of water deficit.



**Figure 2** An holistic approach to future crop breeding. The development of successful elite climate-ready drought tolerant crops for farmer and consumer acceptance will require not just state-of-the-art biotechnology and breeding techniques, but also careful appreciation of the right genetic backgrounds and consideration of a suite of environmental and human factors.

For the reasons highlighted earlier, the complexity of drought stress requires no one single response for crop improvement, but a multipronged approach whereby the whole plant within its agricultural, environmental, and cultural contexts is considered (Figure 2). In this article, we hope to convey the need for and value of this holistic approach within the boundaries controllable by plant scientists, crop breeders, and other key stakeholders who will develop the crop plants of tomorrow.

## 2 Improving Root Responses: Different Routes to Better Roots

Because, for many crops, the fruits or the seeds are the attention centres of agricultural improvement, most current crop selection programmes have focused on shoot traits. However, drought periods in the field affect not only air humidity but also water abundance in the soil, producing changes in the soil environment and properties impacting the root system. Indeed, although low air humidity may often be the first environmental cue detected by the shoot at the start of drought, the root system rapidly detects drying of the soil profile, responds, and relays this information to the shoot (Passioura, 1983).

Roots play crucial roles in soil anchorage, in constituting the soil–plant–atmosphere pathway for water movement in coordination with shoots and stomata, and in absorbing the nutrients required to maintain optimal plant function (Raven et al., 2003). Unfortunately, as most roots are not readily visible, root-related phenotypes have often been ignored, or been harder to follow and characterise, thus impeding this system as a source for crop improvement (Rost, 2011). However, given the root’s essential functions across developmental stages, the researcher’s attention to this plant organ has substantially increased in the last decade. This new-found interest in below-ground traits has also been spurred on by modern phenotyping techniques including fluorescent reporters, low-throughput high-resolution 3D methods such as CT and MRI, as well higher-throughput methods such as RGB, hyperspectral, and ultrawide-band imaging (Bodner et al., 2017; Truong et al., 2018). The knowledge obtained through these analyses and the establishment of other novel experimental methods to explore crop root biology and its responses *in solum* have opened a broader range of possibilities for yield improvement through the optimisation of root systems.

## 2.1 Modification of Root System Architecture

Plant root responses to water deficit occur across its different components, primary and lateral roots, which together with root hairs, constitute the root system. The spatial organisation of this system, known as root system architecture (RSA), also responds to environmental conditions; such that length, thickness, number, position, and angle of these components may diverge depending on soil type and its homogeneity, the amount of nutrients, salts and water, and/or soil temperature, among others (Rost, 2011; Smith and De Smet, 2012). Plant development is intimately associated with environmental conditions, and root development is not an exception. Hence, RSA properties are the result of an integration of environmental cues into root developmental programs dictated by particular genetic information. This constant and close crosstalk leads to an efficient adjustment to the changing environment, which is the basis of plant plasticity. Therefore, the root system is able to cope with abiotic assaults by adjusting different molecular and metabolic processes, eliciting responses to modulate its development, determining its architecture, and preventing damage and/or protecting its integrity and functions (Rogers and Benfey, 2015; Ingram and Malamy, 2010). This establishes the intricacy of the responses that plants use to adjust to the environmental changes that they naturally or agriculturally have to confront to maintain growth, development, and, more importantly, reproduction. We are only just beginning to understand this complexity exhibited by the root system in response to abiotic stresses such as water scarcity.

When drought reaches a field, crop plants must perceive the reduction in water availability, in the air and in the soil, before they can set up the appropriate responses to endure this stress. Under these circumstances, roots have to deal with increasing solute concentrations, which hinder water absorption because water will tend to leave root cells towards the lower osmotic potentials of their surroundings. This phenomenon triggers sensing, transduction, and response mechanisms at different levels, leading to the adjustment of root development, cell metabolism, and defence. At the whole-organ level, changes in RSA have been reported in some crops in response to drought (for further reviews see Comas et al., 2013; Lynch and Brown, 2012; Ye et al., 2018).

Some plant species that have evolved in water-limiting environments show deeper root systems than others adapted to wet conditions. This observation has led to the inference that deeper root systems may be advantageous for survival and reproduction under drought because they can take advantage of the water stored in the deeper soil layers; a typical scenario under gradual water loss. Examples of this can be found from *Arabidopsis* to domesticated crop species. When grown under drought, maize lines with significantly deeper rooting show better subsoil water acquisition, improved water status, and consequently higher yields, regardless of the reduction in the amount of axial or nodal roots. These maize lines also show enhanced stomatal conductance, shoot photosynthesis, and biomass (van Oosterom et al., 2016; Gao and Lynch, 2016). Similar effects have been found in *Setaria*, wild *Zea mays* (teosinte), rice, and soybean (Sebastian et al., 2016; Lynch, 2018; Liu et al., 2018; Henry et al., 2011; Catolos et al., 2017; Ye et al., 2018). Further supporting this, a maize mutant unable to form nodal roots (*rtcs*) maintains better plant water status than the wild-type line (Sebastian et al., 2016). Additional evidence shows that reduced nodal roots and deeper rooting can improve nitrogen acquisition in maize when, under nitrogen limitation, N can be localised in deeper soil; indicating that this phenotype is also associated with effective uptake of soil resources (Saengwilai et al., 2014; Gao and Lynch, 2016).

Crop RSA phenotypes showing few and long lateral roots have also been associated with improved drought resilience. This is consistent with the idea that a larger root system represents a stronger sink, inducing a competition in the plant itself for water and other resources needed across shoot and root growth, thus limiting the growth of other root types (Lynch, 2018; Zhan et al., 2015; Ruta et al., 2010; Comas et al., 2013). Root system formation and maintenance require considerable metabolic investment, corresponding approximately to 50% of photosynthesis per day (Ehleringer and Monson, 1993; Schneider and Lynch, 2018). Therefore, even though lateral roots may be the most effective in water uptake, root architectures with fewer but longer lateral roots are able to search larger soil sections. This target phenotype may, therefore, be more efficient in rhizosphere resource acquisition, in particular for

mobile resources such as water and soluble nutrients. Examples of this have been found in maize, sorghum, and also in some legumes, among other crops (Ye et al., 2018; Zhan et al., 2015; Mace et al., 2012; Rostamza et al., 2013; Polania et al., 2017a).

Additional root phenotypes associated with deep rooting and drought avoidance include growth angle, diameter, and thickness. Growth angle determines direction and distribution of different root types, whereas diameter and thickness traits alter root penetration through deeper soil layers (Vadez, 2014; Wasson et al., 2012; Sperry et al., 2003; Comas et al., 2012; Lynch, 2015). Drought often promotes the formation of fibrous roots and decreases lateral root diameter. These phenotypes have been detected in drought tolerant cultivars from monocotyledonous and dicotyledonous crops, including legumes (Ye et al., 2018; Uga et al., 2008; Henry et al., 2012; Vasellati et al., 2001; Purushothaman et al., 2013; Richards and Passioura, 1989; Salih et al., 1999; Meister et al., 2014; Mapfumo et al., 1994). In soybean, the number of metaxylems has been correlated with drought tolerance, suggesting that this may lead to more efficient water transportation through the roots (Purushothaman et al., 2013; Prince et al., 2017). It has been proposed that aerenchyma formation induced by drought in maize and soybean roots may mitigate the stress caused by water deficit because it participates in more efficient soil exploration (Chimungu et al., 2015; Zhu et al., 2010a; Yang et al., 2012; Niones et al., 2013). Xylem vessel size and number may also be advantageous root traits under low water availability in the soil; thinner vessels among legumes are indicators of higher drought tolerance (Ye et al., 2018; Purushothaman et al., 2013; Prince et al., 2017).

Optimisation of root phenotypes as a target for drought tolerance improvement requires consideration of the differential investment needed for the production of different root cell types. This directs our attention to the cortical parenchyma, the dominant tissue type in primary roots of monocotyledonous crop species in particular. It has been proposed that roots with fewer cortical cells could improve primary root growth, necessary for soil exploration, due to the lower investment required for metabolic activities (Schneider and Lynch, 2018; York et al., 2013; Schneider et al., 2017; Lynch, 2015; Chimungu et al., 2014). When exposed to drought, maize lines with less cortical parenchyma exhibited less respiratory demand, greater elongation of axial roots, improved water status, and superior yield in this adverse environment. Similar findings were obtained for genotypes with higher formation of root cortical aerenchyma, supporting the idea that the transformation of cortical cells to airspaces also permits the redirection of nutrient resources for the development and growth of other plant tissues (Schneider and Lynch, 2018; Chimungu et al., 2015; York et al., 2013; Jaramillo et al., 2013).

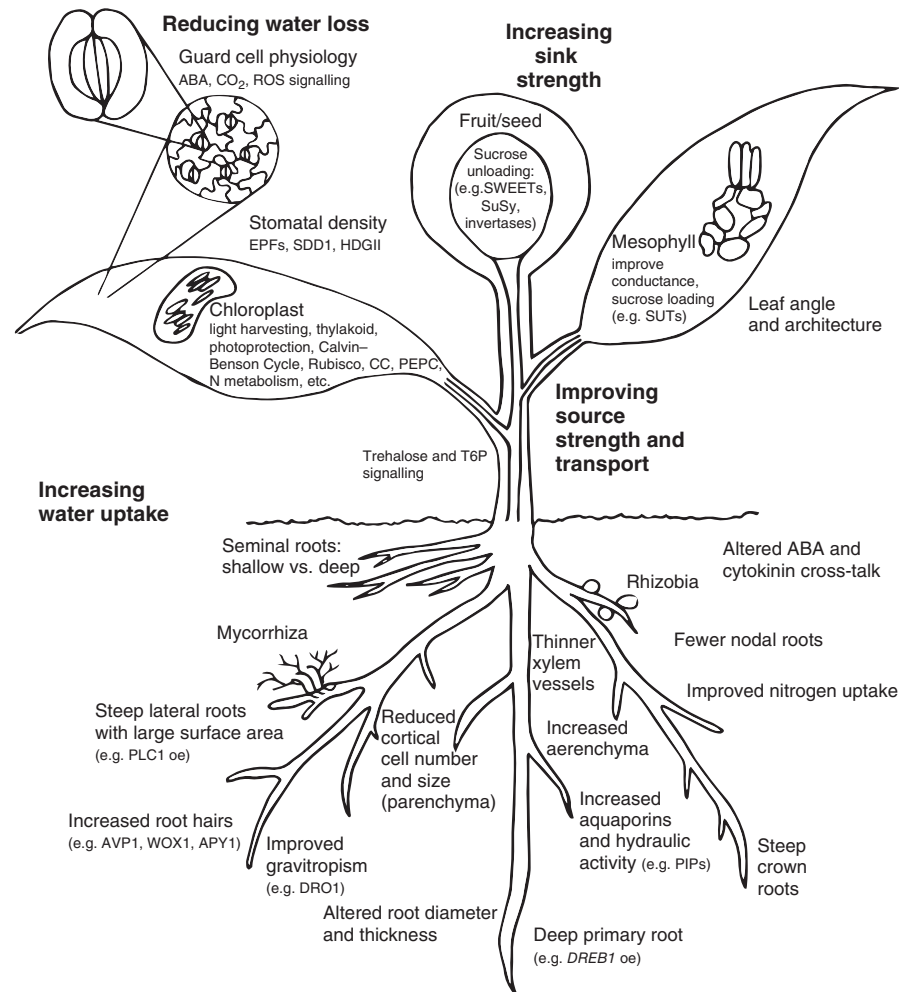
Blum (2015) has proposed two ideotypes of plants based on characteristics of agro-ecological regions and on types of drought. These were named as the 'isohydric', or 'water-saving', and the 'anisohydric', or



'water-spending', ideotypes. The most appropriate ideotype for environments exposed to severe droughts may be the 'water-saving' ideotype, whereas the 'water-spending' ideotype may be optimal in moderate drought stress environments. In this article, common bean genotypes, considered as 'water spenders' and showing high grain yield under terminal drought, present deep roots and higher root vigour (Polania et al., 2017b). However, there are also examples where 'water spender' ideotypes showing vigorous root systems are poorly adapted to drought because they are unable to efficiently remobilise soluble carbohydrates for grain filling (see also Section 2.3 relating to optimisation of water and nutrient transport processes under drought). In addition, it is important to note that some 'water saver' ideotypes may also exhibit deeper and vigorous root systems under drought, denoting the relevance of stomatal control as a crucial mechanism in this adaptation strategy (see Section 2.2 relating to optimisation of stomatal and leaf characteristics under drought) (Polania et al., 2017a,b; Polanía et al., 2009; Rao et al., 2006). Further highlighting the complexity of these responses and potential limitations of simplified ideotype targets, some common bean genotypes considered 'water savers' present only modest root vigour and slower root growth, but combined with a better remobilisation of photosynthates necessary for grain formation and filling, show superior grain yield under drought (Polania et al., 2016, 2017a,b). These traits are of particular importance under terminal drought, when water is needed for reproductive development (Figure 1). These observations highlight the pertinence of considering a suitable combination of root and shoot traits for different drought conditions and soils (Figures 2 and 3).

Even though the rationale and some reports indicate that a deep and vigorous root system might be favourable for crop production under drought, other limitations should be considered. For example, profuse roots may cause localised soil compaction, thus hindering water extraction. Similarly, root systems that show vigorous deep root growth may exhibit a spatial distribution suitable only for soils with specific properties. An additional constraint of deep profuse roots may be found in crops cultivated in areas with strongly seasonal rainfall, which would also need to extract water from surface layers when rains fall, unless they are growing in sandy soils (Vadez, 2014; Comas et al., 2012; Jin et al., 2017; Meister et al., 2014; Koevoets et al., 2016). This again highlights the strong edaphic, geographic, and climatic factors to be considered almost case-by-case in crop improvement (Figure 2). Maintaining both vigorous root and shoot systems in crops subjected to terminal drought would be risky because the shoot's need for water during reproduction and grain filling would exacerbate rapid water depletion from the soil. To complicate matters further, we have also to consider the hydraulic capacity of roots and its relation to water extraction. This is because roots also balance water flow according to shoot water demand and the available water, modulating its apoplastic and symplastic transport. These and other arguments lead to





**Figure 3** Target trait development for future drought-resistant crop ideotypes. To optimise yield under both drought and irrigation agriculture, combining a suite of below- and above-ground traits may be the most successful approach. By improving water and nutrient use through modification of root system, stomatal, and leaf characteristics, and at the same time improving photosynthetic efficiency, carbon transport, and sink strength, we may be able to achieve the next generation of crop plants. For more information about specific gene or trait targets please refer to the main text.

the need for a holistic plant view, considering genotype variability, time and space, and resource availability (Figure 2).

## 2.2 Molecular Targets for Root Drought Responses

The development of optimal crop root systems, not only for plant survival but for improved performance and yields under drought conditions, will be the consequence of the modulation of multiple signalling networks sensing both environmental conditions and endogenous signals. Pyramiding events and/or selecting for several traits will be required for key genetic regulators and signalling factors involved across developmental stages and cell types to promote and/or repress the expression of numerous different genes (Figures 2 and 3). Gene stacking for drought has recently been successful in *Arabidopsis* (Kudo et al., 2018). Model plant systems like *Arabidopsis* have allowed the identification of many of the genetic elements implicated in signalling transduction pathways and regulation of the downstream genes needed for root system development (reviewed in Smith and De Smet, 2012; Wachsman et al., 2015). Different lines of evidence in these model systems have shown that drought sensing and signal transduction in roots is mediated by the crosstalk between different hormone signalling pathways, in particular, abscisic acid (ABA) (reviewed in Rowe et al., 2016; Ullah et al., 2017). ABA synthesis occurs in roots and leaves, and this is boosted by water deficit and other stress conditions (Hu et al., 2016). In root systems, ABA is mainly synthesised in root tips, with a decline in mature roots. ABA accumulation plays a central role during the early response to drought, yet high ABA levels seem not to be beneficial under persistent or severe water scarcity (Sreenivasulu et al., 2012). The correlation between increased ABA biosynthesis and auxin transport to the root tips, followed by lateral root and root hair growth under moderate drought conditions, exemplifies the interplay between ABA and auxin (Rowe et al., 2016). There is also evidence for an interaction between ABA and ethylene in roots exposed to drought, where ABA accumulation is accompanied by ethylene reduction and inhibition of ethylene-mediated elongation and growth (Rowe et al., 2016). ABA and cytokinin crosstalk has also been observed, whereby the ratio between these two hormones is important for signalling from roots to shoots during drought (Ko and Helariutta, 2017; Verslues, 2016). Intriguingly, the overexpression of the isopentenyltransferase (IPT) gene, encoding a rate-limiting enzyme in cytokinin biosynthesis, confers yield improvements in cotton but only if water deficit occurs prior to flowering (Zhu et al., 2018), thus showing the critical importance of the onset and timing of drought (Figure 1).

Despite the vast and valuable research into crop root drought responses, there is still scant information regarding the modulation of these networks to construct a root system with the required properties under drought.

Nonetheless, some of the root system phenotypes described earlier have been correlated with particular genes and phenotypes of their corresponding mutants, mainly in *Arabidopsis*, and, occasionally, in crops. For instance, the ectopic overexpression of the HD-ZIP transcription factor HDG11 leads to high drought tolerance in *Arabidopsis*, a phenotype (*extremely drought tolerant, edt1*) that may be explained by the induction of the expression of cell-wall-loosening protein genes, which in turn leads to the formation of deeper primary roots and more lateral roots than wild-type lines (Yu et al., 2008) (see also Section 3.1 on stomata for further discussion of HDG11 and this mechanism). Similar phenotypes have been found in Chinese kale, rice, poplar, and cotton when the HDG11 transcription factor is ectopically overexpressed (Yu et al., 2013, 2016; Zhu et al., 2016). Transgenic rice plants overexpressing *EDT/HDG11* showed drought tolerance at reproductive stage without a yield penalty (Yu et al., 2013).

Improved yield under drought has also been reported for rice plants with high expression of the *DRO1* (*DEEPER ROOTING 1*) gene, which leads to an increased downward bending of roots, apparently due to an increased gravitropic response in root tips which is correlated with their altered auxin distribution (Uga et al., 2013). The distribution of roots in the soil seems to be linked to the expression of the *DREB1A* transcription factor, which has also been documented in groundnut (*Arachis hypogaea*), where *DREB1A* overexpression produces higher deep root length density (Vadez et al., 2013; Vadez, 2014). Transgenic cotton plants expressing the constitutively active form of the *AtDREB2A* transcription factor (*AtDREB2A-CA*) under the control of the water deficit-inducible *rd29* promoter also show increased total root volume, surface area, and total root length, a root system which tends to grow into deeper soil layers and is associated with better performance under drought when compared to the nontransgenic parent (Lisei-de-Sá et al., 2017). Overexpression of different *NAC* transcription factors in *Arabidopsis*, rice, groundnut, and wheat has led to the formation of root systems with characteristics that conferred to some crops a better performance under drought. Some of the resulting phenotypes include the promotion of lateral root number and length, thicker roots with enlarged stele, cortex and epidermis, larger root diameter resulting from more cells in stele and aerenchyma tissues, and also longer roots and higher root biomass (Meister et al., 2014; Janiak et al., 2016).

Phospholipase C (PLC), another class of regulatory protein implicated in different developmental signalling processes, is also associated with biotic and abiotic responses. Drought and osmotic stress conditions promote the expression of some *PLC* genes, particularly *PLC3* and those involved in PLC-PA (phosphatidic acid) responses (Testerink and Munnik, 2005; Hong et al., 2016). *Arabidopsis PLC3* mutants show phenotypes in lateral root formation and germination, and its overexpression improves drought tolerance not only in *Arabidopsis* but also in maize, canola, and tobacco (Wang et al., 2008; Georges et al., 2009; Tripathy et al., 2012). Similar phenotypes have been

found in transgenic *Arabidopsis* overexpressing *PLC5*, suggesting that these genes may be good candidates for drought tolerance crop improvement (Zhang et al., 2018).

Concerning those genetic elements implicated in the modulation of hydraulic conductivity in the root system, some clues have been uncovered. Some of these are related to aquaporin genes, particularly *PIP1*, *PIP2*, and *RWC-3*, whose enhanced expression in grapevine, maize, and poplar has been associated with an increase in water permeability of the root cortex (Bramley et al., 2009; Laur and Hacke, 2013; Parent et al., 2009; Vandeleur et al., 2009; Meister et al., 2014). In addition, improved root hydraulic conductivity was obtained in tomato and maize plants overexpressing an NCED (9-*cis*-epoxycarotenoid dioxygenase) enzyme involved in ABA synthesis, in which aquaporin abundance increases among other proteins (Parent et al., 2009; Thompson et al., 2007; Chaumont and Tyerman, 2014).

In some crops, constitutive or root-specific overexpression of some potential gene targets may be required to obtain root phenotypes for improved below-ground responses under drought. Such is the case of a group 2 *LEA* gene in cotton (Magwanga et al., 2018), of the rice *AHL1* gene, encoding a protein involved in root development under drought in rice, of the peroxisomal ascorbate peroxidase, which enhances root growth (Zhou et al., 2016), and of the *AVP1* gene, encoding a vacuolar pyrophosphatase, whose high expression in tobacco increases the amount of root hairs (Gamboa et al., 2013).

### 2.3 Linking Root Genetics with Breeding

The massive amount of information available concerning drought responses in crop root systems, in some aspects contradictory, reflects the natural variation and plasticity in populations; consequences of the diverse adaptation strategies selected throughout evolution under different environments, as well as plant species domestication through the selection of traits important to particular peoples and relevant for particular locations (Figure 2). This scenario adds complexity to the choice of particular genes able to confer drought tolerance to crops from diverse regions, together with the fact that their introduction in different backgrounds may lead to unexpected penalties, including yield drawbacks. Under this situation, different strategies have to be adopted, considering quantitative trait loci (QTL) mapping to identify useful root traits that can also be used in marker-assisted selection (MAS) for drought tolerance improvement targeted to specific environments (Collins et al., 2008; Steele et al., 2006; Comas et al., 2013; Selvi et al., 2015). Even though root traits can be difficult to recognise, efforts in this direction have identified QTLs linked to characters including increased root length, root biomass, root number, deep root growth, and seminal root angle or number (Ruta et al., 2010; Giuliani et al., 2005; Ashraf, 2010; Price et al., 2002; Collins et al., 2008; Bernier et al., 2009; Christopher et al., 2013; Courtois

et al., 2009; Hamada et al., 2012; Sharma et al., 2011). In sorghum, it has been possible to identify a nodal root angle QTL associated to grain yield, showing the value of this approach (Mace et al., 2012).

More recently, the ability to sequence complete plant genomes and the accessibility of this information have allowed the implementation of genome-wide association studies (GWAS), which have helped to dissect the genetic basis of some root traits. These kind of analyses have rendered loci to regions of reported QTLs for root traits in rice (Comas et al., 2013; Li et al., 2017b). Interestingly, some of these loci have been associated to root-related genes linked to increased drought resistance, such as *DRO1*, *WOX1*, *APY1*, and *PID* (Wasson et al., 2012; Li et al., 2017b). *DRO1* is involved in the formation of deeper roots by controlling root angle (Uga et al., 2013); *WOX1* and *APY1* both participate in the regulation of root hair formation and root development (Cheng et al., 2016; Liu et al., 2012; Clark and Roux, 2011); and *PID* is implicated in the control of polar auxin transport and in the development of adventitious roots (Morita and Kyoizuka, 2007).

## 2.4 Enhancing Soil Symbioses

In legume crops, a symbiotic association between rhizobial bacteria and root hairs allows fixation of atmospheric nitrogen, providing a more abundant nitrogen source. However, this rhizobial association is often highly sensitive to drought. Most plants transport nitrogen in the form of amino acids, but most tropical and subtropical legumes (including common beans and soybeans) transport nitrogen as ureides. Under drought, these ureides accumulate in the nodules and roots, suppressing nitrogen fixation. Higher grain yield traits under drought correlate with large root diameter, which leads to better nitrogen fixation, possibly due to the establishment of a higher sink strength in the root system (Baral et al., 2016). Tepary bean (*Phaseolus acutifolius*), a species domesticated in arid and semi-arid regions and considered as a 'water saver', shows grain yield above the mean under terminal drought, with a slender and deep root system, despite its low nodule formation capacity and consequent inefficient nitrogen fixation under these adverse conditions (Polania et al., 2017a).

Another crucial microorganism–plant association, the mycorrhiza, is proposed to have beneficial effects on crops during drought, mainly in arid and marginal regions. Although the basis of the advantages offered by this synergistic fungal relationship are not yet well understood, abundant reports indicate that it modifies host plant water relations through mechanisms independent of those implicated in 'classical' phosphorous acquisition (Jin et al., 2017; Quiroga et al., 2017; Augé, 2001). The benefits of this symbiosis have been attributed to the mycorrhizal hyphae system and the production of the glycoprotein glomalin, which are both involved in soil structure modification, allowing higher moisture conservation and better

water uptake through enhanced surface area and soil/mycorrhizosphere chemistry (Comas et al., 2012; Rillig et al., 2002).

It is clear from the above evidence that knowledge generation across different fronts, considering root genetics, physiology, phenotyping, and cellular and molecular biology, is increasingly needed, and that there are many advantages offered by combining these different strategies to improve crop roots in an extremely changeable environment.

### 3 Improving Shoot Responses: Adapting Leaf, Stomata, and Photosynthesis

Reducing transpirational water loss under drought has and continues to be a major target to improve crop responses to water deficit. Plants retain less than 5% of the water they take up through their roots. About 90–95% of the water lost from the plant is lost through the stomata, microscopic valves on the plants' aerial surfaces. Water loss serves several vital functions in plants, moving via osmosis, mass flow, and capillary action from the rhizosphere up through the plant and out of the stomata (and to a minor degree across the waxy cuticle) by evapotranspiration. This process of water loss drives root water absorption, nutrient absorption and transportation, and to a certain extent affects translocation flow. The loss of water vapour from the above-ground parts of land plants as they assimilate CO<sub>2</sub> from the atmosphere for photosynthesis is, therefore, essential for plant productivity and, in agriculture, crop yield. Modern crop development, especially in the twentieth century, has bred for varieties with high stomatal conductance as this correlates strongly with high yields (Roche, 2015; Fischer and Rebetzke, 2018). This selection for higher rates of photosynthesis and higher yields has inadvertently resulted in lower intrinsic water-use efficiency (WUE) in many crops (Koester et al., 2016). This gradual loss in WUE, the amount of carbon gain relative to water use, among many modern varieties has led to crops becoming more drought sensitive and to an increasing reliance on irrigation agriculture to maintain yield gains (Lobell et al., 2014).

The importance of WUE for crop breeding has been understood for over a century (Briggs and Shantz, 1917); however, its use as a target for drought tolerance breeding and improved yields continues to come under scrutiny. There is evidence that if we breed for high WUE alone the yield gains of the twenty-first century shall falter and our crops may become less drought tolerant. Instead, effective use of water (EUW) (in which soil moisture accessed by the plant, nonstomatal transpiration, and soil evaporation are taken into account) can be used to improve yields under water deficit for certain crop ideotypes (Blum, 2009; Polania et al., 2016). Furthermore, as WUE is commonly measured at leaf level, and then extrapolated for daily



integrals or whole-plant estimates its use as a selection target under water stress can be limited, especially for crops with complex canopy architecture (Medrano et al., 2015). Despite these known limitations, WUE remains a favoured measure of plant water use and estimated water deficit tolerance because of its simplicity in the lab and in the field.

### 3.1 Targeting Stomatal Development

Two very simple approaches underlie several biotechnological interventions that could be used to improve WUE, namely optimising stomatal development and optimising stomatal physiology (Figure 3). Both approaches aim to reduce total plant water loss by reducing the pore area available for transpiration. Arabidopsis has provided a solid knowledge base for the genetics underlying stomatal development and physiology, and these molecular mechanisms appear to be highly conserved across land plants (Chater et al., 2016, 2017; Caine et al., 2016), enabling relatively low-risk crop orthologue identification and translational approaches to crop improvement. During above-ground epidermal development, the extracellular EPIDERMAL PATTERNING FACTOR (EPF) signalling peptides EPF2 and EPF1 control density and spacing of stomatal precursors by triggering a mitogen-activated protein kinase (MAPK) cascade to inhibit the bHLH transcription factors that drive stomatal differentiation, SPEECHLESS, MUTE, and FAMA (Hara et al., 2007, 2009; Hunt and Gray, 2009; Lee et al., 2015). Current approaches focus on the overexpression of these and associated negative regulators of stomatal development to reduce stomatal density in crop plants.

Recently, the WUE of poplar (*Populus* species), a tree genus used extensively for manufacturing, as an energy crop for biomass and in ethanol biofuels, was shown to be enhanced by overexpression of native *PdEPF1s* which reduced stomatal density (Wang et al., 2016; Lawson et al., 2014). At low soil relative water contents, *PdEPF1* plants displayed improved carbon fixation and biomass production compared to wild-type controls. Similarly, reducing stomatal density in barley by *HvEPF1* overexpression (Hughes et al., 2017) and in rice by *OsEPF1* overexpression (Caine et al., 2018) have both been shown to reduce water loss and maintain yields under water deficit. Both barley and rice plants with fewer stomata showed attenuated rates of photosynthesis, although with no apparent yield losses. However, *OsEPF1* overexpression phenotypes with more extreme reductions in stomatal density, like wild-type controls, also suffered yield penalties under drought. The reduced stomatal densities in rice also appeared to improve heat stress tolerance, counter to the expected inhibition of evaporative cooling (Caine et al., 2018). As heat stress is generally concomitant with drought stress in the field, any side-effects of stomatal manipulation that reduce evaporative cooling efficiency will need to be carefully monitored. In unrelated experiments, downregulation of *AsAFB2* and *AsTIR1* by



Osa-miR393a also reduces stomata density and increases cuticular density in creeping bentgrass, a turf crop, and enhances heat stress tolerance associated with induced expression of a small heat-shock protein (Zhao et al., 2018). Furthermore, the overexpression of a rice SUMO E3 ligase gene *OsSIZ1* in cotton appears to improve both drought and heat tolerance (Mishra et al., 2017). Therefore, the links between stomatal density, pore water loss, and thermoregulation under water deficit require further careful investigation.

The EPFs transduce their signal by acting as ligands to ERECTA-family membrane receptors modulated by other components including the receptor-like protein TOO MANY MOUTHS (reviewed in Marcos et al., 2016), SERKS (Meng et al., 2015), and a subtilase SDD1 (STOMATAL DENSITY AND DISTRIBUTION 1) (von Groll et al., 2002). Although SDD1's mechanism of action is currently unclear (Pillitteri and Dong, 2013), its overexpression reduces stomatal density. Overexpression of *ZmSDD1* has been exploited to improve drought resistance in maize (Liu et al., 2015) and recently a wild tomato *SDD1*-like gene *SchSDD1* was shown to improve water deficit tolerance in cultivated tomato (Morales-Navarro et al., 2018). However, it remains unclear how *SDD1* overexpression affects seed and fruit development as no harvest or yield data were reported for these species in either studies.

The ERECTAs arguably play a central role in WUE across plants (Masle et al., 2005). There are known associations between ERECTA genes, their expression, and drought tolerance in common beans (Blair et al., 2016) as well as wheat (Zheng et al., 2015). Although the exact mechanisms are still unclear, ERECTAs may also influence plant WUE by reducing stomatal density through a pathway independent of the EPFs, which acts on epidermal cell expansion (Xiang et al., 2017). This pathway, also beneficial in root development (see Section 2.3) (Yu et al., 2013), is putatively driven by EDT1/HDG11-upregulation of *ERECTA*, which likely induces cell endoreduplication and expansion via the transcription factor E2Fa. Indirect manipulation of *ERECTA* through *EDT1/HDG11* overexpression has already been shown to reduce stomatal densities and enhance drought tolerance in several crop species including cotton and poplar (Yu et al., 2016), wheat (Li et al., 2016), alfalfa (Zheng et al., 2017), sweet potato (Ruan et al., 2012), pepper (Zhu et al., 2015), and Chinese kale (Zhu et al., 2016). As these data suggest promising yield data and appear to target both above-ground and below-ground traits, EDT1/HDG11, and its novel pathway may be strong contenders for crop improvement under drought.

One major and as-yet under-utilised resource is the broad natural variation in stomatal densities and patterning across crop genotypes and crop wild relatives. This variation may provide invaluable genetic material for breeding but will rely on improving high-throughput phenotyping and genotyping to

link epidermal traits with water use and their causal genes (Shahinnia et al., 2016; Nunes-Nesi et al., 2016).

### 3.2 Targeting Stomatal Physiology and Guard Cell Signalling

Modifying stomatal function through altering guard cell physiology as an avenue for drought tolerance has many potential molecular targets which interact with numerous endogenous pathways and environmental signals. For these reasons, the scope for improvement is perhaps wider than with stomatal density and could offer more flexibility in whole plant responses under changeable environments. Whereas stomatal density is set upon full leaf expansion, stomatal aperture adjustment is to some extent a continuous process, which can be fine-tuned after maximal leaf growth. A leaf stomatal density optimised for drought may not necessarily support a plant's full yield potential under other environmental conditions or biotic stresses. However, potential drawbacks of targeting guard cell mechanisms also derive from this flexibility; there may have been very strong selection pressures on stomatal physiology in particular varieties, species, and families, which may have driven greater divergence and specialisation of orthologues. Although broad signalling pathways are highly conserved, expansions have resulted in both genetic redundancy and potentially greater risks involved in putative orthologue identification.

ABA-induced stomatal closure is perhaps the most well-characterised plant water deficit response and offers much promise in crop improvement. Guard cell ABA signalling is involved in cross-talk with many other signalling pathways including CO<sub>2</sub> (Chater et al., 2015; Hsu et al., 2018), light (Hayashi and Kinoshita, 2011; Devireddy et al., 2018), and pathogen sensing and responses (reviewed in McLachlan et al., 2014). Considerable expansion of ABA receptors may provide an opportunity to modulate tissue-specific responses among other targets (Park et al., 2015; Okamoto and Cutler, 2018). Directly crop-related improvements of stomatal ABA signalling are few in comparison to the number of Arabidopsis ABA- and drought-related findings, but recently several promising ABA-associated gene candidates have been studied in crops themselves. These include a ubiquitin E3 ligase *PeCHYR1* in poplar (He et al., 2018) and an *abscisic acid, stress and ripening (ASR)* gene *OsASR5* in rice (Li et al., 2017a), both of which enhance drought tolerance through a ROS-mediated ABA stomatal closure pathway, as well as a maize phytochrome-interacting transcription factor *ZmPIF1*, which also improves rice stomatal function and yields under drought (Gao et al., 2018). Mutation or silencing of the *ERA1* gene, *Enhanced response to ABA 1*, encoding a  $\beta$ -subunit of a farnesyltransferase, also holds some promise, having been shown to enhance stomatal ABA responses and photosynthesis under water deficit in canola (Wang et al., 2005), soybean (Ogata et al., 2017), and wheat, perhaps via cross-talk with ethylene signalling (Daszkowska-Golec et al.,

2018; Manmathan et al., 2013). Overexpression of a rice gene *OsTF1L*, which encodes an HD-Zip transcription factor, also causes more sensitive stomatal closure responses, resulting in higher yields due to improved photosynthetic capacity during both vegetative and reproductive stage drought (Bang et al., 2018). While the majority of these recent findings show some positive yield data under water deficit in greenhouse or controlled environment conditions, it remains to be seen how these results translate to the field. Furthermore, and most critically, for any of these modifications to translate to farmer acceptance and commercial viability, the improved photosynthetic capacities reported under stress cannot come with hidden costs under optimal conditions.

### 3.3 Carbon Assimilation, Manipulating the Source

Manipulating stomatal traits, both in terms of stomatal development and stomatal physiology, impact directly on the dynamics of photosynthesis. We are only beginning to understand the degree to which we may be able to alter stomatal traits for improved crop drought resistance without negatively impacting carbon fixation and yield. This inextricable link deserves special attention, for a combination of improved stomatal and photosynthetic traits may greatly enhance crop drought performance (Figure 3). Targeting photosynthesis can increase crop yield potential (Zhu et al., 2010b; Long et al., 2015) when other factors are held constant like photosynthate partitioning and factors influencing sink growth (Long et al., 2006). As plant leaves function as the main site of photosynthesis, improving the production of photoassimilates from carbon fixation in these organs has the potential to dramatically increase the efficiency of all downstream metabolism and biosynthetic pathways in the plant (Siahpoosh, 2015). It is argued that only by improving photosynthesis biochemistry in tandem will higher transpiration efficiency (TE) and WUE not reduce crop productivity (Blum, 2009); however, the rise in atmospheric CO<sub>2</sub> levels may partly compensate for the carbon 'deficit' under higher WUE across many plant species (Drake et al., 2017). Unfortunately, the increasing intensity of future droughts and rising temperatures are likely to reduce these benefits of carbon dioxide 'fertilisation' (Gray et al., 2016).

Biochemically, targeting key steps in the Calvin Benson cycle could increase photosynthetic capacity for improved drought performance. Overexpression of Rubisco alone has not given the desired results in C<sub>3</sub> rice, already saturated with the enzyme (Suzuki et al., 2007), but recently its overexpression in C<sub>4</sub> maize (which naturally contains 70% less rubisco than rice) in concert with Rubisco assembly factor *RAF1*, has enhanced carbon fixation and productivity (Salesse-Smith et al., 2018) and may pave the way for improving photosynthesis under drought. The development of recombinant Rubisco, without or with decreased oxygenase activity and with higher catalytic rates is so far without success (Bathellier et al., 2018),

but increasing the capacity for regeneration of ribulose biphosphate via overexpression of sedoheptulose-1:7-bisphosphatase in crops (Köhler et al., 2017) remains a promising lead for crop improvement under stress (for more detailed reviews of other targets see Long et al., 2006; Sonnewald and Fernie, 2018; Cummins et al., 2018). Photosynthetic resilience to short-term drought stress has also been evidenced in rice seedlings overexpressing C4 *phosphoenolpyruvate carboxylases* (PEPCs) from maize and millet (Ding et al., 2015). Enhancement of chloroplast nitrogen metabolism by overexpression of chloroplastic glutamine synthetases also appears to confer photosynthetic protection and enhanced drought resistance in transgenic rice (James et al., 2018), as well as nontransgenic rice (Singh and Ghosh, 2013) and wheat cultivars (Yousfi et al., 2016; Nagy et al., 2013). Improving photosystem II stability and antioxidant capacity by overexpression of the transcription factor *ZmNF-YB16* has also recently been reported to result in higher maize yields under drought (Wang et al., 2018).

There is evidence that several targets could improve both stomatal and photosynthetic traits at the same time under drought; however, it is important to differentiate between modifications that will provide yield improvements under stress and those that will also improve yield in times of optimal irrigation. For example in drought-resistant wheat, stomatal characteristics which reduce transpiration rate can improve WUE to maintain photosynthetic performance for grain-filling under water deficit (Li et al., 2017c), but this may not translate to competitive yields under normal water supply. The drought- and heat-tolerant cotton obtained by overexpressing the rice SUMO E3 ligase *OsSIZ1* appears to have achieved just that, with higher net photosynthesis, growth, and yields under both water deficit and rainfed field conditions (Mishra et al., 2017).

Two significant and related targets include exploiting the variation that exists in the rapidity of stomatal responses as well as light acclimation amongst species, which could both provide novel targets for improving photosynthesis and water use (McAusland et al., 2016; Salter et al., 2018; Nunes-Nesi et al., 2016; Qu et al., 2016). But it is not just day-time stomatal responses that could play a role in improving crop water use efficiency; reducing night-time transpiration could also be a critical target, as has been demonstrated in grapes (Coupel-Ledru et al., 2016). In addition, crop canopy shape and architecture, although complex traits, have strong potential for increasing efficiency of conversion of intercepted light into biomass (Long et al., 2006), while also improving drought avoidance by altering transpiration and avoiding photoinhibition by avoiding direct sunlight. Many plants, including crop species, already adjust leaf angle (paraheliotropism) and rolling to reduce water loss under stress (Baret et al., 2018; Rosa et al., 1991; Pastenes et al., 2005), and these mechanisms could be enhanced along with shoot architecture. For example, light distribution and interception by horizontal canopy leaves, in crops with three or more

nodes or layers of leaves, are inefficient and wasteful (Long et al., 2006); a better arrangement would be to design more vertical upper leaves and more horizontal lower leaves (Figure 3). These many diverse approaches to improving drought responses of the leaf, the primary site of photosynthesis, including modifying stomatal density, stomatal physiology, and photosynthesis, show the myriad opportunities for crop improvement under climate change.

#### **4 Improving Transport: Linking Source to Sink Under Drought**

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When plants are exposed to drought, both water and carbon dioxide become limiting due to the scarcity of water in the soil profile and due to stomatal closure. The rapid onset of substrate limitation for photosynthetic energy production forces the plant to make major adjustments to carbohydrate allocation (Zamski, 1996), and water limitation may rapidly uncouple carbon fixation from sink growth (Muller et al., 2011). Efficient carbon assimilation in photosynthetic organs (the source) as described earlier, their translocation (loading, transport, and unloading of the phloem) and finally, the strength of the sink (consumer organs such as differentiating tissue, roots, flowers, seeds, and fruits) are, therefore, key points that directly impact on crop productivity (Chang and Zhu, 2017; Sonnewald and Fernie, 2018), particularly under drought. Yield is not necessarily source-limited, and therefore even in the presence of excess photosynthate, drought can result in major sink limitation as the effect on growth can be larger than the effect on photosynthesis (Muller et al., 2011).

Sink organs have been the major site of artificial selection and domestication as they are typically harvested and utilised for food, fibre, and increasingly as a source for renewable fuels (Bihmidine et al., 2013). Since the Green Revolution, crop sink strength, its ability to transport and load sucrose to improve yield, has been indirectly selected for by targeting harvest index traits under watered and droughted conditions. Harvest index is usually used to express the ratio of harvested product (e.g. seed) to shoot dry weight and is used as a proxy for plant carbon balance and reproductive efficiency. Its use in crop breeding under drought from the mid twentieth century onwards has selected for plants that can translate assimilated carbon into yield under stress (Araus et al., 2002). Following this approach in the era of molecular biotechnology could prove very fruitful. In this section, we will focus mainly on those genes and partitioning processes that have already been modified and/or have the potential to improve crop yields under drought stress.

As productivity is the central requirement of agriculture, and not just plant survival, any yield penalties in drought-tolerant crops are not acceptable (Griffiths and Paul, 2017). In this article, source–sink relationships acquire great relevance. Unfortunately, although much effort has been made in exploring this relationship, we are still far from fully understanding source–sink interactions and even further from their correct management (Griffiths and Paul, 2017). One approach involves the manipulation of enzyme activities to regulate source and sink activity. Several recent reviews have explored the engineering of C metabolism enzymes to increase this activity (Zhu et al., 2010b, Valluru et al., 2014), but here we highlight those approaches that may be most relevant in water deficit agriculture.

#### 4.1 Translocation

Improved photosynthesis *per se* may not be sufficient to increase crop productivity, especially under drought, and should arguably be developed in concert with more efficient photoassimilate translocation to the organs of agronomic interest (Ainsworth and Bush, 2011). Recent biochemical and molecular advances in our understanding of cell-to-cell and long-distance partitioning of sugars in plants (Turgeon and Wolf, 2009; Braun et al., 2014; Chen, 2013; Ayre, 2011) have created novel opportunities to manipulate partitioning pathways to enhance yield (Yadav et al., 2015), both under water replete and water deficit conditions.

Sucrose is the principal form of reduced carbon that plants transport long-distance. Sucrose phloem transport can be divided into three sub-processes: phloem loading, long-distance translocation through the sieve elements, and phloem unloading at sink organs. Each subprocess is a potential target to improve crop yields under unfavourable conditions. Sucrose is a relatively large polar solute, requiring active protein transporters (SUTs) for efficient movement across membranes (Yadav et al., 2015). Many SUTs, both symporters and antiporters, have been well characterised and are dependent on the energy released from a proton gradient ( $H^+$ ) (Bush, 1993; Braun and Slewinski, 2009; Ayre, 2011). Some SUTs are localised at the plasma membrane to pump sucrose into cells from the apoplast, whilst others are localised to the tonoplast (Endler et al., 2006; Reinders et al., 2008; Eom et al., 2011). When passive transport suffices, a different kind of sugar transporter, the SWEETs, promote facilitated diffusion (Chen et al., 2010; Chen, 2013; Braun et al., 2014).

#### 4.2 Enhancing Phloem Loading

Constitutive or sink/source-specific SUT expression may be inadequate for improving apoplastic phloem loading, futilely cycling back to the apoplast



for mesophyll cell recovery (Yadav et al., 2015). This inefficient phenomenon has been observed in potato overexpressing spinach *SoSUT1* under the CaMV 35S promoter (Leggewie et al., 2003). To prevent this and ensure constantly high loading rates, tissue-specific SUT expression under heterologous promoters uncoupled from the endogenous regulation has been used (Srivastava et al., 2009), but this manipulation is not yet accurate and may result in unexpected and undesired consequences. For example, endosperm-specific barley *HvSUT1* expression in wheat grains was shown to increase levels of storage protein instead of sugars (Weichert et al., 2010), and tuber-specific expression of rice *OsSUT5Z* in potato resulted in enhanced tuber yield by increasing tuber numbers and not tuber size (Sun et al., 2010). These results suggest that while manipulation of source-leaf phloem loading may energise the entire pathway, it does not promote resource allocation to desired (i.e., harvested) organs (Yadav et al., 2015), and therefore may offer little improvement or yield benefits under drought.

#### 4.3 Phloem Unloading

Sink-specific manipulations may be more fruitful for enhancing yield of specific organs (Figure 3), and sink strength has for a long time been known to play a key role in drought tolerance (Chang and Zhu, 2017). The sink's ability to use available carbon for growth and final crop yield may become severely limited under water deficit, and sink limitation, therefore, may play a far greater role in drought tolerance than previously thought (Muller et al., 2011). However, the sucrose unloading pathway, from the sieve elements to the final sink tissues, may be more complex than phloem loading (Patrick, 1997). The filial tissue is symplastically isolated from maternal tissues, requiring sucrose to cross two membranes (Zhou et al., 2009), involving many SWEETs and SUTs, as well as filial-specific hexose transporters due to the presence of wall invertases (Ruan and Patrick, 1995; Patrick, 1997; Zhang et al., 2006, 2007; Jin et al., 2009; Yadav et al., 2015). Little is known about how hexose transporter modification may impact crop yield, but they have recently been implicated in pathogen resistance in wheat (Moore et al., 2015). Owing to the limited impact of manipulating a single element of the transport system, pyramiding and coupling the targeted expression of genes encoding SWEETs, invertases, and hexose transporters may be more fruitful to improve crop yields (Yadav et al., 2015) (Figure 3).

#### 4.4 Other Key Sink Regulatory Elements

Once sucrose reaches the sink tissue following phloem unloading, it must be either converted into glucose and fructose by invertases or degraded into UDP-glucose and fructose by sucrose synthase (SuSy) for various metabolic,



biosynthetic, or signalling processes (Braun et al., 2014). Invertases are classified into three major groups: cell wall invertases (CWINs), cytoplasmic invertases (CINs) (also found on mitochondria and chloroplasts), and the vacuolar invertases (VINs) (Braun et al., 2014). Reduced invertase activity may cause early seed abortion under drought (Andersen et al., 2002), while CWIN overexpression can lead to increased grain size in rice grains (Wang et al., 2008), and increased fruit size in tomatoes (Jin et al., 2009). Therefore, modulating CWIN activity may have the potential to improve plant productivity and sink strength under unfavourable conditions (Liu et al., 2013). SuSy, which controls the storage phase of seed development, is considered a biochemical marker for sink strength in crops, whose activity has been enhanced through domestication (Braun et al., 2014). SuSy overexpression in cotton results in more mature seeds and cotton fibres (Xu et al., 2012), and SuSy also appears to be instrumental in wheat seedling drought tolerance (Nemati et al., 2018) and terminal drought resistance in common bean (authors' unpublished data).

#### 4.4.1 Trehalose-6-phosphate (T6P) and Trehalose

The promise of trehalose and its associated metabolites for improving crop yields and resilience has recently been reviewed (Paul et al., 2018), but we shall briefly mention its importance and potential. Trehalose and its precursor T6P are important, but low abundance, plant regulators, and signalling metabolites in most vascular plants (Braun et al., 2014). T6P inhibits the catalytic activity of sucrose nonfermenting-related protein kinase1 (SnRK1) and regulates numerous plant development processes (O'Hara et al., 2013; Braun et al., 2014), perhaps playing a central role in whole-plant metabolic reprogramming (Oszvald et al., 2018). There is a strong correlation between trehalose metabolism and signalling with drought tolerance (Garg et al., 2002). Based on the trehalose protective activity observed through *in vitro* assays on proteins under dehydration and its high accumulation upon hyper-osmotic conditions in yeast and some anhydrobiotic organisms (Morano, 2014), the strategy of improving stress tolerance by increasing trehalose biosynthesis has been attempted in several crops, including tobacco (Holmström et al., 1996; Romero et al., 1997; Karim et al., 2007), potato (Goddijn et al., 1997), rice (Garg et al., 2002; Jang et al., 2003), and tomato (Cortina and Culiáñez-Macià, 2005). However, the improved stress tolerance observed has often been associated with pleiotropic growth aberrations which are probably due to altered T6P levels, rather than high trehalose levels (Schluepmann et al., 2003, 2004). Recently, the flower-specific expression of a rice *trehalose-6-phosphate phosphatase* (*TPP*) gene in maize was shown to reduce drought-induced seed abortion and thereby improve yield (Nuccio et al., 2015). The improved yields, even under water replete conditions, show the major role of sink strength and sink limitation (Figure 3), as well as the margin for improvement that exists in our current crop varieties (Oszvald et al., 2018).

## 5 Discussion and Concluding Remarks

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With the advent of gene-editing technologies and improvements in transgenic techniques, we are on the cusp of a revolution in crop plant development. As evidenced by the recent publications showing *de novo* domestication by gene editing (Lemmon et al., 2018; Zsögön et al., 2018; Li et al., 2018), the power to select and mould the plants of our choice is unprecedented in human history. The rich wealth of data available from *Arabidopsis* and other model organisms, combined with functional genomic and transcriptomic resources, now allows almost seamless translational research from the lab to the field. The increasing awareness of the importance of crop wild relatives and the exploration and exploitation of wider gene pools for crop improvement, coupled with the increasing power of molecular breeding based on QTLs, SNPs, and GWAS, have all opened up a vast palette from which we can create distinct varieties based on almost the exact traits of our choosing. For example, SNPs identified through GWAS could be used to inform the development of novel alleles through gene-editing technologies and rapidly confer improved drought tolerance to crops. But we still lack the knowledge of exactly which traits to change for the desired effects. There are major unknowns on this journey, for example interactions with the environment, changing climate, and even consumer tastes and ethics (Figure 2). And the most difficult questions remain unanswered. How do we go about improving crop drought tolerance without trade-offs? Conspicuous by their absence in most of the studies to date are agronomically meaningful field analyses of yield. These data, costly and time-consuming to obtain, and lacking funding support for long-term and multisite studies, will probably remain unresolved for many biotechnological innovations, especially for European science given the recent legal rulings against gene-editing technologies in agriculture and food (Callaway, 2018). Nevertheless, by improving below- and above-ground plant traits, and the signalling and transport between the two, it is possible that many of the trade-offs related to a specific trait can be balanced. In this way, it may be possible to select and/or produce high-yielding crops suitable for a suite of environmental conditions and extremes.

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