Developing drought-resistant cereals

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E-CHAPTER FROM THIS BOOK

Identifying and exploiting genes controlling root system architecture for improving drought tolerance in cereals

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1 Introduction

Understanding the nature of the drought a farmer might encounter is important prior to devising potential solutions, whether they are agronomic or genetic, or both. Each drought scenario should be considered, including the timing and severity of the water deficit, the probabilities of drought occurrence over time, and trade-offs that might have to be balanced when there are costs associated with the expression of a trait or combination of traits that may enhance the resilience of the crop to drought (Tardieu and Tuberosa, 2010; Tardieu, 2012; Comas et al., 2013; Tardieu et al., 2018). As genomic studies increase our understanding of the complex genetic networks that regulate root growth and development (Wachsman et al., 2015; Slovak et al., 2016; Zaidi et al., 2016; Siddiqui et al., 2021), there is increasing focus on root traits as a means of improving yields for water-limited conditions (Koevoets et al., 2016; Voss-Fels et al., 2018; White, 2019; Li et al., 2021; Lynch, 2022). Yet, important questions remain about which combination of root traits confers net economic and environmental benefits to achieve an optimised and sustainable farming

system for drought-prone areas. The list of potential root traits to consider is extensive (Freschet et al., 2021). Ideotypes have been formulated for root system architectures (RSA) that conceivably would be advantageous under certain drought scenarios (Voss-Fels et al., 2018; Li et al., 2022; Rambla et al., 2022), but it is unlikely that such benefits would be observed universally (Lynch, 2018).

2   Root system architecture ideotypes for water-limited environments

The concept of 'designing' root systems based on fundamental biological insight and principles of environmental physics has a long history (Passioura, 1972). For instance, in regions with terminal drought, crops are reliant on stored soil moisture to complete grain filling. Excessive consumption of water too early in the season leaves too little moisture available in later stages and limits grain filling (Wasson et al., 2012). Removal of soil water by the crop can be limited by reducing transpiration via stomatal closure at high vapour pressure deficit (VPD; Messina et al., 2015), reducing water transport from roots to shoots by decreasing hydraulic conductivity of the vascular system (Richards and Passioura, 1989) and by decreasing the biomass of active roots in the soil volume (Lynch, 2018). Selection for genotypes with small diameters of open xylem elements in seminal roots was suggested as an effective way to increase axial resistance of water flow in roots and thereby conserve soil moisture (Richards and Passioura, 1989). Much less attention has been given to the role of phloem pressure unloading in maintaining root growth, which is critical for the delivery of assimilates to expanding cells (Stanfield and Bartlett, 2022; Zhou et al., 2022). Root systems that can reach deeper soil layers to access greater volumes of stored soil water at depth also appear to be beneficial (Lopes and Reynolds, 2010; Rich et al., 2015), but with little disadvantage in well-watered crops, as shown in wheat (Manschadi et al., 2008; Christopher et al., 2013; Maccaferri et al., 2008; Maccaferri et al., 2016; Li et al., 2019), maize (Hund et al., 2009; Lynch, 2013) and rice (Uga et al., 2013; Pandit et al., 2020). In sorghum, QTLs for the stay-green phenotype (*Stg* loci) under drought co-located with QTLs for root:shoot ratio and nodal root angle (Borrell et al., 2014). Nevertheless, the metabolic cost of producing deep roots and the potential yield drag need to be balanced with the benefit accrued (Li et al., 2022). In one study, there was a yield advantage to steeper, deeper roots in water-limited environments, but a yield loss in well-watered environments, compared to lines with wide, shallow roots (el Hassouni et al., 2018). Another study did not observe any cost to above-ground biomass or yield in deep-rooted lines (Severini et al., 2020). The relationships between root angle and yield were context-dependent in barley (Robinson et al., 2018) and durum wheat (Alahmad et al., 2019). As Passioura

(1983) pointed out: 'There is no point in a droughted crop investing a parcel of assimilate in its roots if the extra water thereby obtained does not allow the shoots to at least replace the assimilate so spent'. A mathematical formalisation of the concept led to the definition of 'root system efficiency', which expresses the carbon investment in root mass for water acquisition (or transpiration per unit leaf area), per unit dry root biomass (van Oosterom et al., 2016; Li et al., 2022).

The realisation of the genetic potential for deep root growth depends on soil conditions that are permissive to growth (Rich and Watt, 2013). In some regions, hardpans, bedrock, low/high pH, sodicity, salinity, high levels of aluminium or boron, and root pests and pathogens may pose a greater limitation to root proliferation than the genotype (Richards et al., 2014; Hobson et al., 2022). Furthermore, following the extraction of subsoil water by roots, it is necessary that the soil profile is recharged by precipitation or irrigation in time for the subsequent crop if there is to be any advantage gained by deeper root growth (Ahmed et al., 2018).

2.1 Genes controlling root growth angle and roots in deep soil layers

Based on simple geometry, deeper soil layers can be reached more quickly by the root system when the growth trajectory of roots is steeper, compared with a shallow root angle that would promote greater proliferation of roots in shallow soil layers (Lilley and Kirkegaard, 2011). A number of studies have discovered loci that control root angle, such as a QTL on chromosome 6A in durum wheat that has a major effect on seminal root angle (Sanguineti et al., 2007; Maccaferri et al., 2016; Alahmad et al., 2019). This QTL also collocates with the QTL cluster controlling osmotic adjustment in roots (Condorelli et al., 2022). Recent research has revealed a suite of genes that are part of the regulatory network that controls the gravitropic response that governs the angle at which roots grow away from the central root axis (Toal et al., 2018). This 'gravitropic setpoint angle' (GSA) relates to the tissue responsiveness to auxin gradients established within the elongation zone of root tips (Roychoudhry et al., 2017). This is offset by a separate antigravitropic mechanism that counteracts the positive response to the gravity stimulus, thus maintaining the growth trajectory. In Arabidopsis, genes regulating GSA are members of the *TIR1/AFB* auxin response mechanism. In barley, two mutants that show extreme gravitropic response (steep seminal root angle) are *egt1* and *egt2*, identified from a Morex TILLing population. TILLing mutants are an effective reverse-genetics resource for the functional characterisation of candidate genes. Wheat orthologues of *egt1* and *egt2* were also identified recently in both tetraploid durum and hexaploid bread wheat. The *egt1* locus is part of the antigravitropic mechanism,

encoding a TUBBY-like F-box protein that regulates cell wall remodelling during growth resulting in cortical cell wall stiffening, which counteracts the cell wall loosening in epidermal cells that occurs as roots respond to gravity-induced auxin fluxes (Fusi et al., 2022). The *egt2* orthologue encodes a STERILE ALPHA MOTIF protein that regulates the positive gravitropic response (Kirschner et al., 2021). In the proposed model, root bending towards gravity is caused by an egt2-regulated increase in peroxidase activity in epidermal cells, resulting in hydroxy radical-induced wall loosening (Somssich et al., 2016) and enhanced elongation of epidermal cells on the side of the root in opposition to the gravity vector. This particular phenomenon of the establishment of root growth angle progresses from activity at the gene level in small groups of cells in the root tip to the relative distribution of root system biomass throughout the soil profile, with implications for the access to and uptake of soil water.

In the case of *egt1* and *egt2*, the mode of action is on cell wall mechanics, but the differential growth process clearly involves many processes, each of which is regulated in turn by different components of the regulatory gene network. For example, synthesis, transport and response to hormonal signals (Ubeda-Tomás et al., 2012); electrophysiological setpoints controlling solute transport; osmotic potentials that drive water fluxes for the maintenance of turgor pressure (Ober and Sharp, 2003); homeostasis of secondary messengers Ca^{2+} and reactive oxygen species (Voothuluru et al., 2020), etc. Each process represents a multitude of potential gene targets for selection and manipulation by breeding.

Inadvertent selection for root angle may have resulted from pleiotropic effects of the *VRN1* gene that controls the expression of winter/spring types in wheat and barley via a MADS box transcription factor (Voss-Fels et al., 2017). In European and Australian wheat lines, the winter allele of *VRN1* is associated with a narrow root angle, although the molecular mechanism is undetermined.

2.2 Root hairs

Root hairs are the tubular outgrowths from specialised epidermal cells that greatly expand the total surface area in contact with the soil (Vissenberg et al., 2020). Experiments comparing wildtype and mutants exhibiting defective root hairs showed that root hairs were vital for water uptake under water-limited conditions (Carminati, et al., 2017; Klamer et al., 2019; Marin et al., 2021), but perhaps less so in the absence of stress (Dodd and Diatloff, 2016).

Root hairs are also important for rhizosheath formation (the soil that adheres to the root surface), which is mediated by ABA and auxin effects on root hair elongation under soil drying (Zhang et al., 2021). Rhizodeposition, or the exudation of mucilage and other compounds by the root, also plays a role in forming the rhizosheath (McCully, 1999; Holz et al., 2018). The rhizosheath

combination of soil, root hairs and mucilage forms an essential bridge of hydraulic connectivity between the root surface and the bulk soil (Carminati et al., 2017), allowing the transfer of water (Cai and Ahmed, 2022) and nutrients (Ober and Parry, 2011; Kang et al., 2022). A subset of wheat lines of the Avalon × Cadenza doubled-haploid mapping population showed significant genotypic variation for rhizosheath size and mucilage composition (Marr, 2020).

Details of the complex gene networks controlling root hair growth and development are being unravelled (Salazar-Henao et al., 2016), partly through genetic dissection and functional characterisation of mutants that show defective root hairs (Chmielewska et al., 2014). While this is a useful approach, mutant lines are not suitable for breeding; donor lines that show advantageous alleles are required for genetic gain. As there is a multitude of potential gene targets, focus on the master regulators in the control pathway is warranted. An example in wheat is the gene *TaRsl4*, which encodes a bHLH transcription factor, modulating root hair length (Han et al., 2015). Interestingly, in allopolyploid wheat, there is a genomic asymmetry in *RSL4* expression, such that the homologue on the A chromosome shows greater expression than the B or D genomes, perhaps due to differential chromatin modification. Using sequence information from the conserved regions of the gene, a survey of allelic variation across various germplasm pools should reveal haploblocks that are associated with longer root hairs. Forward genetic screens of diversity panels via genome-wide association study can reveal epistatic interactions with other loci and also identify individuals representative of long root hair haplotypes, providing donor material for introgression into elite backgrounds for evaluation within a breeding programme. This kind of haplotype-led breeding is useful for allele stacking (Brinton et al., 2020). Other potential gene targets for root hair density, which is usually correlated with length (Nestler et al., 2014), are *RSL2,* a transcription factor (Han et al., 2017)); *RHD3*, a GTPase responsible for vesicle trafficking (Shan et al., 2005); *AUX1*, an auxin transporter in rice (Giri et al., 2018) and *RTH5*, an NADPH oxidase (Nestler et al., 2014).

2.3 Allocation of biomass to roots and its spatial distribution

It would seem intuitive that greater root biomass, and therefore, greater increased soil water extraction, would confer an advantage during drought. However, the yield response depends greatly on how root biomass is distributed in space and time in relation to the phase of development of the crop and rainfall patterns. For instance, lines with smaller root biomass were beneficial in terminal drought situations, as they consumed less soil water during the pre-anthesis stage than lines with greater root biomass (Figueroa-Bustos et al., 2020). This was also reflected in a modelling exercise and subsequent tests of the model (van Oosterom et al., 2016). There is evidence that root biomass was

inadvertently selected against as a result of breeding for selections for heading date (Voss-Fels et al., 2018). In European bread wheat, the major haplotype variant controlling heading date has two flanking haplotypes controlling root biomass, and selection against the late heading data allele resulted in linkage drag, reducing root biomass simultaneously. A relatively rare haplotype variant was associated with larger root biomass, which was linked to two candidate genes that play roles in root growth: expansin (catalyses wall loosening) and endo-beta-mannanase.

Other studies also show changes in RSA traits over time as a consequence of breeding and selection for yield under high input conditions and high plant population densities (compared with ancient practices). For example, examining lines representing 100 years of wheat breeding in Switzerland, there was a negative correlation between year of variety release, plant height and rooting depth, although this was observed only under well-watered conditions, not drought (Friedli et al., 2019). Similarly, the year of release was negatively associated with root system size in wheat lines from several decades of breeding (Fradgley et al., 2020). Zhu et al. (2019) concluded that 'Green Revolution' alleles for semi-dwarfing and plant yield were selected more strongly than rooting depth. It is interesting to speculate that as plant population densities increased with intensive modern agriculture, the competitive ability of older varieties was indirectly selected against, in favour of genotypes that were less sensitive to neighbours, as shown in a study comparing an old and modern line of wheat (Fang et al., 2011). At the root level, this could manifest as a greater tolerance to the co-exploration of roots from different individuals within a set soil space (Fang et al., 2013; Hecht et al., 2019), which could more rapidly deplete soil moisture reserves.

While a smaller root system size may be beneficial in terminal drought environments, in other environments with episodic periods without rainfall and intermittent drought eventually relieved by rainfall, loss of yield potential due to stress can be minimised by more efficient extraction of stored soil water. In an analysis of winter wheat crops in the UK, it was shown that the mean root length density (RLD) in soil layers 0–30 cm from the surface typically exceeded the critical value of 1 cm root length per 1 cm^3 soil volume (there was superfluous root production), whereas at 50 cm and below, RLD was less than 1 cm cm^{-3} (inadequate root biomass) (White et al., 2015). A critical RLD of 1 cm cm^{-3} was shown by modelling and empirical measurement to allow the full extraction of available soil water from a typical mineral soil (Gregory, 1989). However, where certain spatial patterns of root biomass distribution exhibit trade-offs with unstressed yield potential, and drought occurrence is unpredictable, a stochastic approach to finding the optimal balance in RSA (Tardieu et al., 2018), or a highly plastic root system (see later) is necessary.

There has been little systematic testing of root biomass traits in a uniform genetic background with sufficient numbers of lines. To achieve this, a major QTL for root biomass in wheat was discovered on chromosome 5B (Voss-Fels et al., 2017), and a KASP marker was developed for this locus (Makhoul et al., 2020). Marker-assisted backcrossing into an elite background was used in conjunction with a simple visual scoring method to select lines that diverged in seedling root biomass (Rambla et al., 2022). In combination with these lines, additional lines that contrasted in seminal root angle were also backcrossed into the same background, such that sets of introgression lines comprising four combinations were developed: high vs. low biomass combined with wide vs. narrow root angle (Fig. 1). These materials are being used in field trials to assess the potential contribution of each combination in different environments. The combination of phenotypic selection and speed breeding (Watson et al., 2017) allows the rapid development of near-isogenic lines (NILs) in breeder-relevant backgrounds to assess relative trait values.

2.4 The ability of roots to penetrate soils

From the discussion so far, it is clear that for the root system to become established within the three-dimensional soil space, root apices must extend through the soil matrix. In the field, soils are often inhospitable, and high soil strength or compaction can create a formidable barrier. Many soils also harden as they dry, presenting plants with an additional challenge during drought (Whalley et al., 2008). In fact, the extent of soil drying, caused principally by the removal of water by roots, can be used as a proxy measure for genotypic differences in yield in water-limited environments by using a simple soil penetrometer (Whalley et al., 2008).

In order to continue the exploration of the soil, roots must either penetrate soil layers, grow around areas of high soil strength until roots are able to penetrate or find cracks, fissures or 'biopores' within the soil peds. Biopores
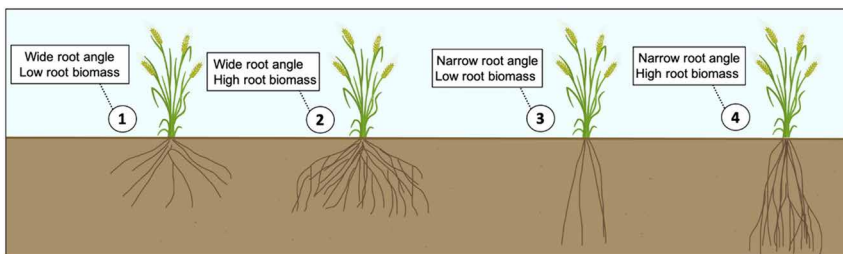

Figure 1 An example of different root ideotypes that can be generated by combining different genetic loci controlling root angle and root biomass. Used with permission from Ober et al. (2021) under the Creative Commons Licence http://creativecommons.org/licenses/by/4.0/.

are natural holes created by roots of previous crops that have subsequently decayed or by earthworms. It has been shown that most of the roots that grow to depth in soil with high penetration resistance do so via these pores (White and Kirkegaard, 2010). Genotypes with thicker roots have greater tensile strength and are often better able to penetrate hard soils, as shown in maize (Chimungu et al., 2014) and rice (Clark et al., 2008). Increased wall thickening in multiseriate cortical sclerenchyma (Schneider et al., 2021) or increased radial expansion of cortical cells is related to the accumulation of ethylene as a result of reduced gaseous diffusion through the soil around the root tip (Pandey et al., 2021). A mapping study revealed a MEI2-like RNA binding protein co-located with a major QTL peak for MCS formation (Schneider et al., 2021). Ethylene-induced deposition of lignin may also increase root stiffness. Alternatively, thin roots are better able to enter small cracks between peds than thicker roots.

Using micro-computed x-ray tomography, Atkinson et al. (2019) found that in compacted soil, 76% of roots changed direction (altered trajectory of growth), compared with 21% in loose soil. A peculiar behaviour of roots is that growth often follows a spiral and waving pattern called circumnutation; it has been suggested that this helps in obstacle avoidance (Fang et al., 2013) and increases the chances that a root tip will encounter a crack or macropore (Taylor et al., 2021). Key regulatory genes in rice, a histidine kinase (*OsHK1*) and an auxin influx transporter (*OsAUX1*), may prove useful breeding targets for problem soils. By using wax layers of variable hardness to mimic strong soil, one study found that the number of root axes that managed to penetrate a hard layer was simply related to the total number of root axes produced by a genotype (Whalley et al., 2013). The wax layer method was used to map QTLs for penetration ability, but many QTLs with small effects limited marker development (Botwright Acuña et al., 2014). Other features that assist with penetration ability are anchorage via root hairs (Bengough et al., 2016) and lubrication via mucilage exudation (Oleghe et al., 2017).

3   Root-derived hydraulic and hormonal signals

Regulation of the balance between root and shoot (R:S) growth plays an important role in maintaining yields during drought. A wild emmer introgression into durum wheat improved transpiration efficiency through alteration of R:S, and restricting transpiration to periods of the day when VPD was low (Bacher et al., 2022). Root growth can continue at water potentials (ψ_w) that completely inhibit shoot growth, indicating fundamental differences in how the tissues of above-ground and below-ground structures respond to drought (Sharp and Davies, 1979) (Fig. 2). This can be viewed as an adaptive mechanism that limits shoot biomass and leaf area, which is susceptible to transpirational water losses, and partitions more assimilate to root growth, needed for water acquisition.
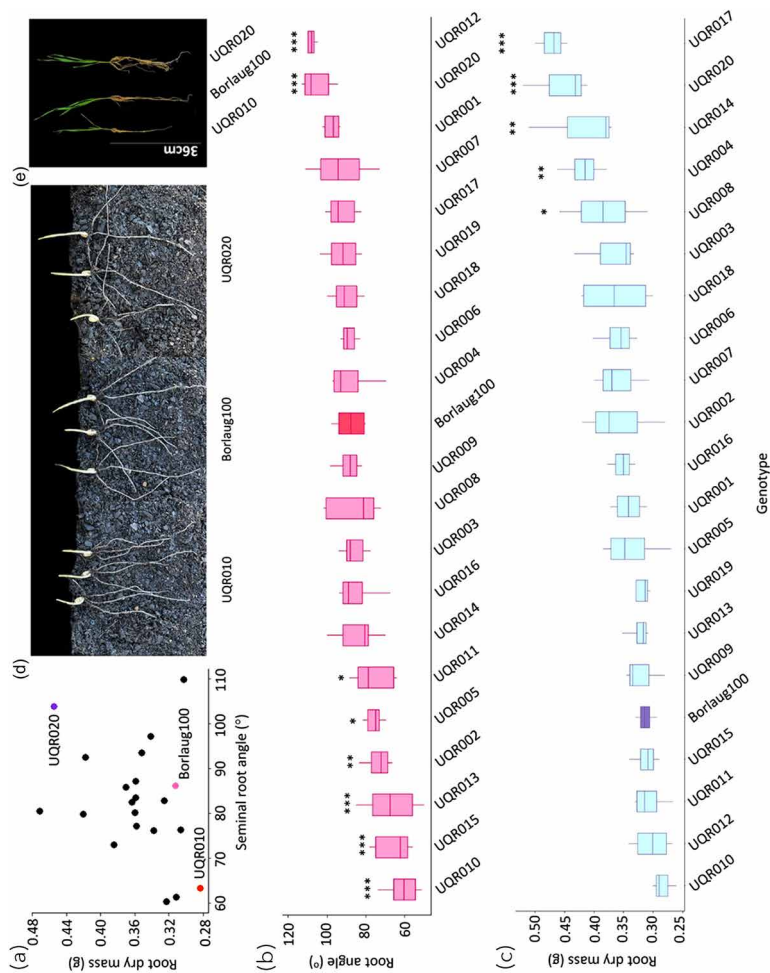
Figure 2 Phenotypes of wheat introgression lines selected for contrasting seminal root angle and root biomass. Lines are $BC_2F_4:F_5$ in a Borlaug100 background. (a) A plot of 20 lines diverging in root biomass and root angle. (b) Boxplots representing the seminal root angle and (c) root dry biomass for each introgression line. Genotypic means of lines were compared with Borlaug 100 using Fisher's protected LSD test (*, ** and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively). (d) Comparison of the seminal root angle between Borlaug100 and the introgression lines UQR010 (narrow) and UQR020 (wide). (e) Plants typical of the root biomass phenotypes of UQR020 (high) and UQR010 (low) compared with Borlaug100. Used with permission from Rambla et al., 2022.

Using the maize primary root as a model system, studies have shown that in low ψ_w media, the maintenance of the growth of cells in the apical region of the elongation zone involves complex interactions of many factors. For instance, growth depends on the production of abscisic acid (ABA), which keeps a check on the growth-inhibitory effects of ethylene; the accumulation of solutes for osmotic adjustment (Condorelli et al., 2022); cell wall modification via ROS and wall loosening enzymes, which allows root cells to elongate at diminished levels of turgor pressure (reviewed in Ober and Sharp, 2013). A key target in maize suggested by a combination of kinematic growth analysis and root zone-specific RNAseq is oxalate oxidase, which plays a role in H_2O_2 production (Voothuluru et al., 2020; Kang et al., 2022).

Some of the earliest responses that trigger these changes occur at the cell membrane, where low ψ_w induces hyperpolarisation of the membrane potential via upregulation of H^+-ATPase activity (Ober and Sharp, 2003). Under prolonged exposure to low ψ_w, the ABA-dependent homeostatic setpoint of the membrane potential is adjusted to a more negative value, maintaining a greater driving force for cation uptake, such as K^+, important for osmotic adjustment along with organic compounds such as proline (Bhaskara et al., 2015).

3.1 Regulation of plant hydraulic conductivity

While production of ABA may be necessary for root growth maintenance at low ψ_w, ABA that enters the transpiration stream via the xylem can lead to inhibition of the growth of leaves (Saab and Sharp, 1989), decreased stomatal conductance, and changes in hydraulic conductivity (Huntenburg et al., 2022). Thus, ABA produced in roots and leaves can act as a non-hydraulic signal but can also induce hydraulic effects, with different responses in different tissues in different environments (e.g. night vs. day; low vs. high VPD; (Tardieu et al., 2010).

ABA and other signalling factors can affect the hydraulic conductivity of roots via modulation of aquaporin (AQP) activity, proteins that facilitate water transport across the cell membrane (PIP gene subfamily) and the tonoplast (TIP gene subfamily; Maurel et al., 2021). Xylem vessel properties (discussed earlier) affect axial root conductivity and suberin deposition (Calvo-Polanco et al., 2021), and AQP affect radial conductivity (Maurel and Nacry, 2020). Transpiration and hydraulic conductance are closely coupled by bi-directional signalling between roots and shoots (Huntenburg et al., 2022). In the early stages of soil drying, root AQP expression is down-regulated and water channel activity is modulated via phosphorylation/dephosphorylation to reduce hydraulic conductivity, hence conserving moisture (Javot and Maurel, 2002; Kapilan et al., 2018). A simplified summary of a large volume of research is that up-regulation of AQP activity in

roots to increase root hydraulic conductivity, and a decrease in AQP activity (perhaps via expression of different tissue-specific isoforms or differential post-translational modification) in shoots to decrease transpiration rates could help maintain favourable water status of plants during drought (Shekoofa and Sinclair, 2018). In terminal drought environments, down-regulation of AQP activity could decrease hydraulic conductivity and conserve soil moisture.

3.2 Hydraulic lift in mixed cropping and agroforestry

We have seen that improving crop production in dry environments entails complexity on many levels, beyond merely the genetics of the plant, involving also the physics and biology of the soil environment. An additional level to consider is the cropping system. Compared with conventional monocrops, utilisation of cover crops is now common practice in many areas, and intercropping and agroforestry may expand beyond smallholder farming to large-scale operations. In addition, there is growing interest in varietal mixtures rather than pure stands. In such systems, roots of different species or varieties share the same soil space (Kemper et al., 2022), yet we understand little of how these interactions may benefit the overall resilience of the mixed crop during a water deficit. In the section earlier, we mention how the suberisation of roots controls radial hydraulic conductivity, limiting the loss of water to dry soil from mature root tissues. However, there is evidence that roots do lose water to the soil (Bormann, 1957) and that nearby roots from the same or different plants can utilise this water (Neumann and Cardon, 2012). An example of 'hydraulic lift' or upward redistribution was shown in an interplanting arrangement of alfalfa and maize, where deeper roots of alfalfa supplied water to the roots of maize in upper soil layers, extending the period of survival during drought (Corak et al., 1987). Mechanisms of this 'reverse flow' are not well understood (Caldwell et al., 1998), but it would be surprising if the rectification of water transport was not tightly regulated by the plant. Roots exhibit a curious tendency for non-random 'swarming' behaviour (Ciszak et al., 2012), such that neighbouring roots are often closely appressed, or clumped together, particularly in soil pores (White and Kirkegaard, 2010). In Arabidopsis, roots can apparently avoid obstacles, but grow towards roots, with a preference for the same genotype (Fang et al., 2013). While this may not be a theoretically efficient distribution of root biomass, it may assist water transfer from one part of the root system/soil profile to another.

4 Plasticity of root growth

It is well known that plant form is genetically programmed, yet the final shape and directionality of growth also depend on environmental signals, some of which trigger various 'tropisms (Darwin, 1880). So too, the underground plant

form follows a pre-determined genetic pattern, but the expression of many root traits can be modulated by the heterogeneous environment of the rhizosphere (Hodge, 2009). The sensitivity of plant tissues to abiotic stresses and the manner in which this information is transduced by cellular machinery to effect changes in developmental patterns are also encoded in the genome and/or epigenome (Lloyd and Lister, 2022). Some interesting and useful examples of this plastic behaviour that impinges on drought tolerance are discussed later.

4.1 Biomass allocation patterns

In a field study that compared isogenic wheat lines that differed only in the wheat-rye 1RS translocation, the 1RS lines showed greater root biomass and yield than the background line without the translocation under well-watered conditions (Ehdaie et al., 2012). However, under water deficit, the line lacking the translocation increased total root biomass in response to stress, whereas the root biomass response to stress was negative in 1RS.1DL or neutral in 1RS.1BL and 1RS.1AL. In a year with mild stress, the positive plastic response was associated with a smaller reduction in yield loss than the 1RS lines, whereas in a year with greater stress intensity, the line with the plastic response showed a greater yield loss, presumably due to greater allocation of root biomass in shallow layers that exhausted limited water supplies, and shifted partitioning of assimilates away from shoots to roots. The region controlling plasticity was located on 1BS using homologous recombinant lines (Ehdaie et al., 2012).

   In rice, QTLs associated with total root length (TRL) were not consistent across well-watered and droughted conditions, and QTLs for plasticity (the relative change in TRL across environments) did not co-locate with QTLs for TRL (Kadam et al., 2017), indicating separate levels of control. Another rice study discovered single-nucleotide polymorphisms (SNPs) related to root biomass plasticity (albeit in a small panel of lines grown in rhizoboxes), which were correlated with yield stability. The alleles were contributed by donor lines adapted to marginal environments and conferred greater root plasticity to an elite line grown in high-yield environments (Sandhu et al., 2016). To identify loci associated with root plasticity under fluctuating soil water conditions, a panel of inbred lines was created from a cross between the recurrent parent Nipponbare and a line that showed enhancement of root branching in shallow soil layers during drought (Owusu-Nketia et al., 2018). One of the NILS showed similar rooting patterns to the recurrent parent under continuously well-watered conditions, but greater lateral and nodal root length under drought, which was associated with greater stomatal conductance and grain yield.

   In maize, QTLs for root trait plasticity across environments did not co-locate with QTLs associated with the traits in individual environments (Schneider et al., 2020). For example, SNPs related to lateral root length and branching density

were located at different chromosomal regions for the plasticity of expression, compared with trait expression in the separate well-watered and stressed environments, except for one common SNP on chromosome 10 that co-located with a candidate gene encoding an ethylene response transcription factor.

4.2 Hydrotropism and hydropatterning

For a time it was debated whether hydrotropism (root bending) or hydropatterning (root branching) – which leads to the proliferation of roots in patches of soil with greater matric potential – was merely the net result of biomass allocation due to slow growth in drier soils, or was the result of directionality of growth and developmental patterning towards moisture (Giehl and von Wirén, 2018). Carefully designed experiments have shown that root cell elongation on different sides of the root does indeed respond to favourable gradients in hydraulic conductivity and water potential of the external medium (Bao et al., 2014), although they may not be necessary (Lind et al., 2021). Regulated by opposing auxin gradients established within the root via PIN transporters (Robbins and Dinneny, 2018), the growth trajectory responds simultaneously to the gravity and water potential vectors (Morohashi et al., 2017), and likely other signals as well (Knight, 1811). Thus, water potential gradients can cause growth to deviate from the genetically determined GSA (mentioned earlier) and follow a new trajectory, directing the placement of roots where there is greater resource availability.

The initiation and growth of lateral roots are additional plastic responses to local soil environmental conditions. Roots growing through a large soil macropore produce lateral roots only where the root is in contact with the wall of the pore and repress the formation of lateral roots where the root is in contact with the air space within the pore (Bao et al., 2014). Inhibition of lateral root formation is dependent on the action of ABA (Orman-Ligeza et al., 2018), which represses the auxin pathway factor ARF7 in Arabidopsis (Orosa-Puente et al., 2018). The absence of water stimulates the modification of the ARF7 protein by sumoylation, which then blocks the recruitment of auxin factors to the 'air side' of the root. On the 'wet side' of the root, auxin-responsive genes such as *LBD16* are free to initiate asymmetric cell division of lateral root founder cells in the pericycle (Babé et al., 2012).

Unlike most axile roots, lateral roots can exhibit determinate growth: cell production from the meristem ceases after a period of growth, defining their final length. However, the genetic programme that determines the duration of development can be modulated by external water potential. In maize, lateral root growth can continue for an additional 3 days under mild water deficits compared with well-watered plants, a response that is genotype-dependent (Dowd et al., 2020). In another study, mild water deficit had no effect on the

linear frequency of lateral roots along the primary or seminal root axes but did increase the final length of first-order lateral roots (Ito et al., 2006).

4.3 Developmental instability, cryptic variation and noise

The RSA can be determined by the angle, velocity, duration and branching of root growth, which respond to both genetic and environmental signals. While plasticity can provide an adaptive or yield advantage, stability of developmental patterns – buffering the phenotype against every tiny or momentary change in the environment – is also vital, and plants manage some kind of balance. In addition to relatively predictable phenotypes due to genetics or environmental responses (plasticity), there appears to be an element of randomness, or noise in many developmental processes. This injection of stochasticity into the system also affords a level of protection against unpredictable environmental challenges at the individual and population level as a 'bet-hedging' strategy (Forde, 2009). Intriguingly, the level of developmental instability (plasticity and noise) that is tolerated appears to be under genetic control in plants (Sangster et al., 2008) and may be mediated in part by chromatin remodelling rather than changes in DNA sequence (Lloyd and Lister, 2022). Changes in phytohormone levels or sensitivity may directly affect chromatin modifiers to transduce environmental stresses to alterations in root meristem maintenance and developmental patterning (Xiao et al., 2017; Maury et al., 2019).

In the constant search for novel variation for breeding improvements, geneticists debate whether cryptic variation – revealed through alteration of chromatin remodelling that can affect epigenetic buffering of development programs – can lead to heritable changes to the epigenome lasting more than a few generations before they reset (Hauser et al., 2011; Bloomfield et al., 2014). It is unclear if the net effect of stress-induced changes to the epigenome is agronomically beneficial (Lloyd and Lister, 2022). Important questions are to what extent increased plasticity and/or developmental stability in root development are desirable in certain environments, and if these alleles or epialleles are amendable to improvement through selective breeding.

5   Root–microbe interactions

5.1 The rhizosphere microbiome

The rhizosphere microbial community and plant roots form complex interrelationships that are rich territory for scientific exploration and potentially exploitation to benefit agriculture and ecosystem services. There is evidence that arbuscular mycorrhizal associations can promote drought tolerance in wheat (Lehnert et al., 2017). Drought-induced changes in root-associated bacterial communities can have positive and negative effects (Aslam et al., 2022), partly

due to the severity of the stress (Naylor and Coleman-Derr, 2017). Benefits to the plant may come from recruiting bacteria that produce growth-promoting hormones (Rolfe et al., 2019) or inhibition of ethylene production (which inhibits root growth) via ACC deaminase (Sayer et al., 2021). The microbiome is supported by carbon and nitrogen substrates exuded by the host plant roots. Metagenomic analysis of the rhizosheath soil isolated from individuals of the Avalon × Cadenza mapping population of wheat showed differences in bacterial and fungal diversity and abundances, which may be related to genotypic differences in the compositional profile of mucilage (Marr, 2020). In future, it may be possible to engineer solutions for droughted conditions by soil inoculation of selected beneficials, in combination with plant genetics that favour a microbial balance that helps protect the plant. However, some evidence suggests that modern cultivars and intensive agriculture management practices have reduced the rhizo- and endosphere species richness (Kavamura et al., 2019).

5.2 Beneficial endophytes

The root endosphere microbiome may also contribute to drought tolerance by the composition of non-mycorrhizal endophytic species hosted by the plant tissues. For instance, drought during early development resulting in severe stress enhances *Actinobacteria* in millet (Simmons et al., 2020). The endophytic fungus *Acremonium coenophialum* in tall fescue promotes tiller survival and recovery from drought (Elemi and West, 1995). A study in rice showed that the host genotype affected the endo-fungal community composition (mostly the Ascomycota subphylum Pezizomycotina), which was associated with enhanced drought tolerance in the field (Andreo-Jimenez et al., 2019). Wheat plants inoculated with Ascomycetous fungal isolates isolated from the roots of field-grown durum wheat resulted in greater seed yield compared with endophyte-free plants (Hubbard et al., 2014). However, these are complex experiments to conduct, and conclusions from controlled environments can be inconclusive (Decunta et al., 2021).

6   Role of roots in supporting yield stability across water regimes

Farmers and breeders traditionally focus on yield potential as the important metric of a variety's performance, but stability across locations and years is becoming increasingly important as climate variability increases with global warming. The need to breed varieties with greater climate resilience and therefore yield stability may increase the focus on the optimisation of RSA (Topp, 2016). Perhaps it will be revealed that some loci that associate with stability co-locate with loci controlling root traits (Mickelbart et al., 2015). Analysis of the natural allelic variation for root traits and stability will inform the haplotype

diversity within pools of germplasm (Qian et al., 2017), which will help breeders choose the best lines for crossing in order to stack beneficial alleles in progeny lines (Brinton et al., 2020).

Varietal performance is usually dependent on interactions with management practices and biotic and abiotic factors in a defined location, over a specific time period (Annicchiarico, 2002). In this context of genotype × environment interactions, yield resilience or static stability can be understood as the insensitivity of a cultivar to environment influences and achieve its yield potential unperturbed (Eberhart and Russell, 1966). This definition does not, however, allow for beneficial interactions that may increase yield in favourable environments, which requires a dynamic view of stability. The concept of yield stability is not new and yet, despite its agronomic and commercial value, there is little agreement on how it should be defined, measured or implemented in practice (Lin et al., 1986; Reckling et al., 2021).

At a physiological level, biomass production and how it is allocated is thought to contribute to yield stability across a range of environments (Weiner et al., 2021). Increased allocation to root biomass in superficial soil layers is typical of cereal crops in arid and semi-arid climates (Wang et al., 2014; Palta et al., 2011; Hamblin and Tennant, 1987). While there are plausible explanations for the advantage each trait previously described would confer to yield stability, the manipulation of root biomass and root growth angle (see Section 2) may pose the most obvious solution. One hypothesis is that in temperate climates a variety with narrow root angles and increased root proliferation in the subsoil would reduce the probability of yield losses at water-limited sites (thus contributing to yield stability), but in semi-arid environments, reduced root distribution in upper soil layers may lead to more consistent yields (Yang et al., 2022; Fang et al., 2021). This suggests that genetic material with narrow root angles such as the *egt1* and *egt2* mutants (see Section 2.1) and introgression lines with high root biomass (see Section 2.3) would have greater yield stability, when compared with the wild-type or recurrent parent, respectively. Experiments to test this hypothesis are underway.

Perhaps, though, the key to realising stable yields in cereal crops across water regimes may not be held by one ideotype, but instead by a combination of root traits. Varietal mixtures (see Section 3.2) comprising contrasting but complementary RSA could more efficiently occupy the soil volume, and therefore in the context of a plant stand (rather than an individual plant) could strike an optimum balance between biomass allocation, soil water extraction and grain yield. The idea that varietal mixtures could support yield stability was introduced alongside the term itself (Eberhart and Russell, 1966), and more recently mixtures have found renewed focus in efforts to improve the sustainability of 'regenerative' farming systems.

7 Breeding for root traits to improve drought tolerance

7.1 Ask breeders what they need

There is no shortage of published papers which suggest that their trait or gene of interest will be useful for breeders. Very few of these traits or genes have been incorporated into active breeding programmes (Passioura, 2012). Jackson et al. (1996) considered this disconnect between fundamental plant science and breeding and arrived at some suggestions, which are still relevant today, particularly for root traits. To find utility in a breeding programme, fundamentally traits should have high heritability, show good genetic correlation with yield and/or sustainability metrics in target environments, be amenable and affordable to selection on a large scale, be characterised in relevant elite material in the field, be identified in useful donor lines representative of major or novel haplotypes, etc. (Richards, 2006). Breeders need no convincing that roots are important and have been overlooked in breeding. But they need to know what to select for, a way to make those selections, and evidence for the impact of those selections on performance in the field in each predicted target environment.

Research on seedling traits in controlled environments or containerised experiments can be useful but does not fully address the earlier needs, as juvenile traits do not always reflect trait expression in more mature plants in the field (Bai et al., 2019; Rich et al., 2020). Measurement of roots in the field at
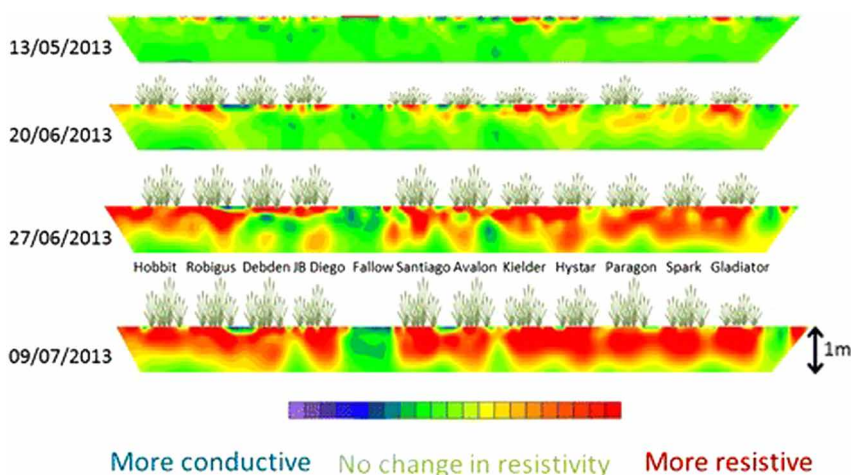

Figure 3 Progressive soil drying shown via time-lapse images created from inversion of electrical resistance tomography measurements in the soil underneath field plots of different wheat varieties. Colours indicate relative changes in soil resistivity after the baseline date 23 April 2013 (red: greater resistivity; green: no change; blue: smaller resistivity). The position of plants in each plot along the ERT transect is indicated, as well as the central bare fallow plot. Used with permission from Whalley et al., 2017.

a medium scale can be accomplished via soil coring and shallow excavations (shovelomics) but is laborious and time-consuming, limiting the throughput capacity (Tracy et al., 2020). Therefore, non-invasive proxy measures that relate to rooting behaviour have been investigated. Two such methods show some promise: inferences about soil water extraction patterns by roots can be made using canopy temperature measurement via thermal remote-sensing (Pinto and
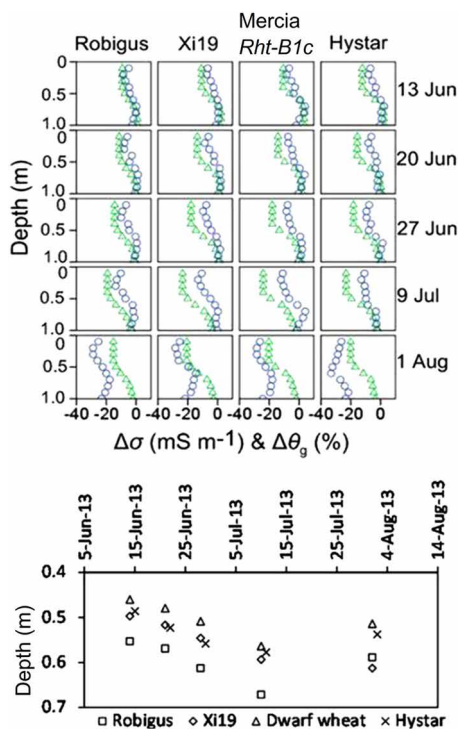

Figure 4 Top: The change in electrical conductivity ($\Delta\sigma$) (baseline date 14 May 2013) over time determined from electromagnetic induction (EMI) measurements (triangles) and the change in gravimetric soil moisture content ($\Delta\theta_g$) (circles), plotted against depth from the soil surface. Negative values indicate a relative decrease in soil moisture content. Different wheat genotypes are indicated at the top. Mercia *Rht-B1c* is an introgression line showing an extreme dwarf phenotype (but despite small shoot biomass, showed similar patterns of soil water extraction to standard varieties). Hystar is a hybrid wheat but did not demonstrate deeper rooting or greater water extraction than other varieties. Robigus is the only variety in this set containing the 1RS.1BL chromosomal translocation from rye. Bottom: Changes in the inflection point of the measured $\Delta\sigma$ over time in each genotype. The inflection point relates to the soil depth where soil moisture content changes rapidly from low to high water content, roughly indicating the rooting front as the root system progresses downward. Measurements were made during a period of little rainfall and therefore plants were removing stored soil moisture over this period. Symbols are the mean of four replicate plots. Modified and used with permission from Shanahan et al. (2015) under the Creative Commons Licence http://creativecommons.org/licenses/by/4.0/.

Reynolds, 2015; Li et al., 2019) and adaptation of electromagnetic induction methods (Figs. 3 and 4) (Whalley et al., 2017; McLachlan et al., 2021).

7.2 Validation: do small phenotypic changes make a difference?

Like many quantitative traits, differences in RSA between elite lines drawn from major germplasm pools are often subtle and difficult to detect. Greater differences can be observed comparing exotic material and agronomically improved lines, but with such diverse sets, the contribution of root traits to yield, drought tolerance or effective use of water can be surmised based only on correlative evidence. Validation of trait value can be better done using NILs that differ only in the locus in question. Even though genotypic differences in trait characters may be numerically small, the impact can be significant. For instance, a deeper root system that can mine an extra 10 mm of subsoil water can translate to an additional 600 kg grain/ha, using an average water use efficiency of 60 kg/ha/mm (Kirkegaard et al., 2007). In wheat, root dry matter at 1 m depth comprises only 3% of the total root biomass, which seems almost negligible, but during a dry period, these roots can contribute 20% of the plant's evapotranspiration (Gregory et al., 1978).

Genotypic differences in root growth angle also can be numerically small, but important. For example, simplifying the root system to a cone (with the point at the root/shoot junction) and applying some geometry, the result of a 10° reduction from 55° (from vertical) to a more narrow angle of 45° is that the root tips reach a depth of 1 m 27 days earlier, assuming a constant trajectory and growth rate of 1.2 cm/day (three-dimensional root simulation models would produce a more realistic result). In an environment where crop phenological development is racing against the onset of terminal drought, quicker access to deep water stores could be vital. There is evidence that a shift in root angle can be achieved quickly through speed breeding (Richard et al., 2018) and that a more narrow RSA can produce a yield benefit in droughted environments, as discussed in the previous section.

Models suggest that a small increase in root hair length from 0.4 mm to 0.8 mm could increase P uptake by 52% in dry soils, compared with a 26% boost in well-watered soils (Wissuwa and Kant, 2021). Field trials have supported the value of root hairs in droughted conditions (e.g. Vetterlein et al., 2022), but these experiments commonly involve comparisons with mutants exhibiting defective root hair development. More research is needed to identify useful donor lines within natural variants and to test the model predictions in a near-isogenic background.

While there is pressure on crop scientists and breeders to deliver step-changes in performance characteristics of cereals to keep pace with the demand of a growing world population, we see that even small changes in the expression of certain traits can have a significant impact.

8 Future trends in research

A recent survey outlined the current research needs of the root biology community (Delory et al., 2022). Chief among those highlighted by the respondents was high-throughput phenotyping methods (Atkinson et al., 2019; Tracy et al., 2020) and a better understanding of the relationship between traits and function.

8.1 Common genetic panels for community-wide phenotyping

The creation of common panels of appropriate genetic materials in elite backgrounds that can be widely shared for research – and with publically available genotype data – might advance understanding faster than traditional correlative experiments that compare small sets of highly diverse lines that are unique to each study (Uga, 2021). Some such sets already exist, such as the Global Durum Panel, various MAGIC populations and NAM panels. Some biparental mapping populations have been used for many studies, such as the Avalon × Cadenza doubled-haploid winter wheat population, with published data ranging from seedlings to the field.

8.2 Root image analysis

Much root phenotyping is not done on roots but on images of roots. Due to efforts by many groups over a number of years, there is a selection of different software applications that automate or semi-automate root image analysis (Falk et al., 2020). Recently, these tools utilise machine learning methods to increase the speed and accuracy of feature identification and quantification (Pound et al., 2017; Wang et al., 2019; Yasrab et al., 2019; Teramoto and Uga, 2020; Bauer et al., 2021; Narisetti et al., 2021; Seethepalli et al., 2021) While some packages require a level of manual curation during the process, or familiarity with artificial intelligence methods to train software to new species or background conditions, newer methods allow non-expert users to train the software quickly using smaller numbers of annotated images (Smith et al., 2022). For analysis of older plants with more complex RSA, and the inevitable overlap and occlusion of root segments, additional care must be taken to obtain accurate phenotype data (Lobet et al., 2017).

8.3 Models for simulating root growth, development and function

There have been a number of advances in the development of models that simulate root growth and function, and these have become more accessible as commonplace computing power increases. Still, simulation models are under-utilised, and as the accuracy and functionality of these models increase, their incorporation into research should help underpin selection and breeding strategies (Lynch, 2018). For instance, functional-structural models such as OpenSimRoot (Postma et al., 2017) and CPlantBox (Zhou et al., 2020) can be used to predict interactions between RSA and nutrient and water uptake, particularly when coupled with soil hydrological models (Tron et al., 2015; Ruiz et al., 2020). Using the whole-plant APSIM maize model, Hammer et al. (2009) showed that RSA (water acquisition) had a greater effect on grain yield than canopy architecture (light interception) in high-density planting with adequate moisture availability. In particular, there was a yield advantage of steeper root angles (25° from vertical) vs. wider angles of 45° in non-stressed environments. Models that incorporate management practices at the farming system level (e.g. Cooper et al., 2021) provide a more holistic view of the potential impact of genetic manipulation of RSA (Thorup-Kristensen and Kirkegaard, 2016).

Other topics introduced above show promise for greater exploration and exploitation in the future. For instance, it is important to better understand the genetic control of RSA plasticity and how genes that control plasticity can be incorporated into improvement strategies (Suralta et al., 2018; Schneider and Lynch, 2020; Schneider et al., 2020).

8.4 Roots and the rhizosphere microbiome

An exciting area for new discovery is the interaction between host plant genetics and the rhizosphere microbiome, aided by technical advances in metagenomics. Harnessing the beneficial effects of soil microbes that can improve the resilience of crops to drought shows promise (Hong et al., 2022). However, we require a greater understanding of the nexus between RSA, root exudates, physical soil properties, soil tillage and crop management practices such as fungicide use, legacy effects in a crop rotation, etc., to provide a holistic view of how practical improvements could be made (Kawasaki et al., 2016; Marr, 2020; Herms et al., 2022). In future, the genetics of the crop and the microbiome could be (naturally or artificially) engineered (Gopal and Gupta, 2016; Yu and Hochholdinger, 2018). Evidence suggests that selective breeding for agronomic traits has indirectly affected the composition of the microbiome, so it follows that this also could be done in a targeted manner (Kavamura et al., 2019; Tkacz et al., 2020).

The research summarised in this Chapter has largely focussed on the major cereal crops, and we have not drawn on a large body of information from other species. A challenge for the future is to support root research and genetic improvement of RSA in other cereals such as millet and other crop species that would be characterised as 'orphan' or neglected. These crops receive less R&D funding but can be vital for small-scale production systems or can be further developed to diversify rotations in farming systems of industrialised countries.

9   Conclusion

The literature cited here illustrates the extensive body of information that supports improvements in roots and the rhizosphere to increase productivity in water-limited conditions. The list of QTLs and candidate genes for root traits is growing rapidly (Kulkarni et al., 2017; Klein et al., 2020; Li et al., 2021). Many of the fundamental principles have been understood for some time: RSA and physiology converge with soil hydraulics to define water acquisition and supply to the shoot, while shoot growth and transpiration converge to modulate the volume of water drawn from soil stores via atmospheric demand (Boyer, 1985). We will no doubt eventually see new varieties released that have genetically modified root systems, or varieties with novel root phenotypes derived using gene editing. These are only techniques that augment conventional breeding methods; the same fundamental questions apply in matching root traits with environments and the usual attention that must be paid to pleiotropic effects or 'off-target' events, which may be deleterious or beneficial.

After the briefest acquaintance with roots in the field, it becomes apparent that phenotypic plasticity to macro- or microenvironmental variation, coupled with developmental instability defines the phenotype as much or more than the fundamental genetic program, and that RSA is not uniform even within a constant genetic background. For those interested in selective breeding for root traits, this cannot be safely ignored. Rather, taking this into consideration, the lessons for crop improvement may be to courageously embrace the noise rather than finding clever statistical methods of partitioning it out of the way, to quantify phenotypic variation to characterised environmental cues rather than treat 'environment' as a nuisance and to employ the complexity of epigenetics rather than exploiting only the simply inherited phenotypes.

The stochastic nature of root trait expression and drought occurrence must always be taken into account when designing solutions. And to deliver those solutions, open and active dialogue between plant biologists, geneticists, breeders, agronomists and farmers must be constantly fostered.

There is no single crop improvement strategy that will fit all situations, particularly with regard to RSA ideotypes (Clarke and McCaig, 1993).

Ideotype breeding may result in highly specialised varieties for targeted environments (van der Bom et al., 2020), while the provision of a 'toolbox' of donor lines carrying a variety of alleles controlling different RSA types (Rambla et al., 2022) will allow breeders to mix and match for different environments differing in resource availability, or for wide adaptation. It is unclear how many robust molecular markers may eventually appear in the toolbox that will be implemented in a marker-assisted selection of root traits; at the moment, there are very few. It is also uncertain if genomic selection methods could be applied to RSA if the requirement is for large training sets of root phenotype data from the field. It can be argued that attention to yield stability across locations and years is as important to farmers as yield potential. Optimising root function may play an important part in reducing volatility in yields that compounds the volatility in market prices and weather that cause farm businesses to struggle.

10   Where to look for further information

The International Society of Root Research (https://www.rootresearch.org/) is a good place to find more information about current research on roots, and the ISRR hosts a conference every three years. Web portals to access software for root modelling and image analysis are available, such as https://www.quantitative-plant.org/. Although peer-reviewed journal articles are the best place to find the most recent advances in root research, there are many oft-cited books on roots, such as 'Plant Roots: The Hidden Half' and the classics that have well-worn pages: Weaver's 'Root Development of Field Crops', Böhm's 'Methods of Studying Root Systems', and Smit et al's Handbook on Root Methods, to mention a few:

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