

REVIEW PAPER

Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops

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Abstract

Wheat yields globally will depend increasingly on good management to conserve rainfall and new varieties that use water efficiently for grain production. Here we propose an approach for developing new varieties to make better use of deep stored water. We focus on water-limited wheat production in the summer-dominant rainfall regions of India and Australia, but the approach is generally applicable to other environments and root-based constraints. Use of stored deep water is valuable because it is more predictable than variable in-season rainfall and can be measured prior to sowing. Further, this moisture is converted into grain with twice the efficiency of in-season rainfall since it is taken up later in crop growth during the grain-filling period when the roots reach deeper layers. We propose that wheat varieties with a deeper root system, a redistribution of branch root density from the surface to depth, and with greater radial hydraulic conductivity at depth would have higher yields in rainfed systems where crops rely on deep water for grain fill. Developing selection systems for mature root system traits is challenging as there are limited high-throughput phenotyping methods for roots in the field, and there is a risk that traits selected in the lab on young plants will not translate into mature root system traits in the field. We give an example of a breeding programme that combines laboratory and field phenotyping with proof of concept evaluation of the trait at the beginning of the selection programme. This would greatly enhance confidence in a high-throughput laboratory or field screen, and avoid investment in screens without yield value. This approach requires careful selection of field sites and years that allow expression of deep roots and increased yield. It also requires careful selection and crossing of germplasm to allow comparison of root expression among genotypes that are similar for other traits, especially flowering time and disease and toxicity resistances. Such a programme with field and laboratory evaluation at the outset will speed up delivery of varieties with improved root systems for higher yield.

Key words: Architecture, drought, genetics, gravitropism, molecular markers, phenotyping, vigour.

Introduction

Rainfed wheat production in many parts of the world is dependent on stored soil moisture. Here we explore which root traits are most likely to be valuable for improving water uptake to increase yield, and discuss efficient ways to select for these traits in breeding. This paper defines an

approach to breeding that can be used in any system where root traits, in conjunction with management, can be used to overcome environmental constraints on growth. This paper focuses on selecting for desirable root system traits for production systems where deep water at the end of the

season is likely. Examples of relevant production systems are the summer-dominant rainfall regions of India, Australia, and southern Africa where water is very limited, but also ranging to more favourable rainfed systems such as in Europe where deep soil moisture may also be available. We note where the traits discussed will be ineffectual or detrimental in alternative environments.

Plant breeders generally shy away from selection for root traits as they have a low heritability, and expression varies with soil type and rainfall (Cooper *et al.*, 1999a, b; Tuberosa *et al.*, 2002; Malamy, 2005). Plant breeders generally assume that direct selection for yield will indirectly select varieties with the optimum root system for delivering the highest yields. This appears to be the case with maize breeding in the USA, where annual genetic yield gain of 77 kg ha⁻¹ has been achieved at the optimum plant density for the varieties grown (Duvick *et al.*, 2004). Modelling suggests that the narrowing of shoot and root architectures in higher density, earlier-sown cropping systems underlies yield gains in these environments, which have relatively high, predictable rainfall during the growing season (Hammer *et al.*, 2009). It is possible that more directed selection for specific root architecture traits could enhance yields in dryland cropping regions. Evidence for this comes from modelling studies for wheat in Australia where it has been shown that selection for deeper, more effective roots could significantly improve capture of water and nitrogen (Manschadi *et al.*, 2006; Asseng and Turner, 2007; Lilley and Kirkegaard, 2011).

Trait-based selection and breeding complements more empirical breeding approaches for yield. Trait-based selection has advantages in that it ensures there is appropriate genetic variation in breeding populations for selection progress to be made. It is also expected to have advantages in terms of selection methodologies using molecular markers. A more complete discussion of the advantages of trait-based selection is found in Richards *et al.* (2002, 2010). Trait-based breeding for root traits is an order of magnitude more difficult than for most above-ground traits. To overcome this, fast laboratory-based selection methods for root traits need to be developed that are related to phenotypic expression in the field. Furthermore, it may be possible to develop a proxy screen of an easily observed and quantified trait that reflects what is happening below-ground. Then, if required, molecular markers that account for a significant proportion of the variability in the root trait may also be identified and developed from these screens, and used within breeding.

This paper will focus on root developmental traits. We recognize, however, that root traits that overcome biotic and abiotic constraints are critical to maintaining root length, function, and water capture, and are first order targets in breeding programmes for rainfed conditions (Passioura, 2006). Organisms that feed on roots, such as fungi, termites, nematodes, and aphids, severely reduce crop performance in dry environments by reducing rooting depth and root proliferation (Audebert *et al.*, 2000). Similarly, toxic levels of microelements such as aluminium (Kochian, 1995; von Uexkull and Mutert, 1995) and boron (Jefferies *et al.*, 2000)

can restrict root growth. A focused breeding approach has been successful in overcoming some of these constraints. Breeding programmes have developed molecular markers for biotic resistances, such as resistance to cereal cyst nematode (Ogbonnaya *et al.*, 2001) and root lesion nematode (Williams *et al.*, 2002), to improve water uptake. Incorporating traits conferring resistances to toxicities have also improved productivity (Tang *et al.*, 2001, 2002; Delhaize *et al.*, 2004; Munns *et al.*, 2006). Overcoming subsoil constraints by direct genetic selection for tolerances to physical and chemical constraints such as extremes in soil strength and density, pH, salinity, and toxicities allows morphological root traits to contribute to deep water uptake, and thus are an integral part of a genetic improvement programme for roots (Yambao *et al.*, 1992; Passioura, 2006; Botwright Acuna *et al.*, 2007; Haling *et al.*, 2010).

We also recognize that agronomic practices can greatly increase storage and conservation of water in a cropping system, and can improve the health, growth, and function of the crop, which can significantly interact with root traits to improve yield (Watt *et al.*, 2005; Hammer *et al.*, 2009; Passioura and Angus, 2010). Practices that increase the conservation, storage and access to water are best combined with new varieties with root traits to capture that water (Kirkegaard and Hunt 2010).

Contribution of deep soil water extraction to grain yield

Wheat production in India and Australia represents a cross-section of global spring wheat production. High-yielding irrigated wheat production and low-yielding rainfed wheat production systems occur in India. In Australia, summer-dominant rainfall patterns occur in the northeast grading to winter-dominant patterns in the southeast, with Mediterranean systems in the south and west. Although the focus is on those regions with summer-dominant rainfall and stored soil moisture, the value of deep soil water in ameliorating water deficits is found in many environments including more favourable environments such as in the UK (Dodd *et al.*, 2011) where winter wheat predominates.

Many wheat production systems could benefit from improvement of the storage of soil moisture (through management) (Hunt and Kirkegaard, 2012) and soil moisture exploitation (through root genetics). There are examples of this potential in India and Australia. In India, rain falls almost entirely in the summer, during the monsoon season when crops such as rice or sorghum are grown or the land is fallowed. All wheat is grown in the winter when it is dependent on water stored in soil after the monsoonal rains plus any supplemental irrigation. 'Rainfed' Indian wheat typically is irrigated once before sowing to allow the crop to germinate and emerge, and then relies entirely on water stored in the soil. Today most rainfed wheat in India is in the Central and Peninsular regions, and accounts for ~30% of India's total production with little access to irrigation. The trend in Indian agriculture is towards less water for

irrigation, and storing and use of deep water, either summer rains or irrigation, will become increasingly important.

Most wheat grown in Australia is rainfed. Like India, it is grown in the winter. In the northeast cropping region, most rain falls in the summer and wheat relies largely on moisture stored in the soil after a preceding summer crop or fallow. In the southeast, rainfall is highly variable in amount but it is generally evenly distributed through the year. It is common for water to be stored deep in the soil at the time of sowing, resulting from the preceding spring and summer fallow as well as good weed and stubble management (Hunt and Kirkegaard, 2012). In the southwest, most rain falls in the winter during the season, and thus stored water at sowing is a less important source of water for wheat, although rapid root growth, deep into the soil during the season may increase capture of water and nitrogen, especially in sandy soils (Anderson *et al.*, 1998; Asseng *et al.*, 2001; Liao *et al.*, 2004, 2006).

The value of targeting the capture of deeper soil moisture with selected root traits in a breeding programme is 2-fold. First, the farmer can measure how much soil moisture is stored and to what depth at the beginning of the season. Farmers can store water through pre-season practices, such as choice of pre-season crop, fallow, or irrigation (e.g. when irrigation water is available or cheaper, in the case of India). Farmers can then minimize evaporation of that water through weed and stubble management (Hunt and Kirkegaard, 2012). Once stored beyond the evaporation zone, it becomes a known source of crop water, while the in-season rainfall is unpredictable at the time of sowing.

The second value of deeper soil moisture is that its uptake generally coincides with grain development when crops are vulnerable to terminal drought (Passioura, 1983). Water use at this time has a very high conversion efficiency into grain (water-use efficiency) as vegetative growth has finished and all photosynthate is used for grain growth. Most of the increase in yield from late season subsoil water use is due to increases in the harvest index (ratio of grain to shoot weight) of the crop (Passioura and Angus, 2010). The high value of deep water was demonstrated in independent controlled-environment and field studies. A comparative study of two wheat genotypes, a standard variety (Hartog) and a deep and more densely rooted variety (Seri), grown in large root boxes showed that greater root length in deeper soil layers contributed to increased yield by allowing more water extraction during grain filling (Manschadi *et al.*, 2006). Modelling based on the apparent extraction efficiency of the Seri genotype suggested that each additional millimetre of water extracted after flowering generated an extra 0.55 t ha⁻¹ of grain yield (Manschadi *et al.*, 2006). Using rainout shelters in the field and drip irrigation, it was shown that 10 mm of subsoil water absorbed between depths of 1.35 m and 1.85 m after anthesis would increase grain yield by 0.62 t ha⁻¹, equating to 59 kg ha⁻¹ for every additional millimetre (Kirkegaard *et al.*, 2007). Hunt and Kirkegaard (2012) recently re-evaluated the benefit from stored out-of-season rainfall at 37 sites throughout southern Australia under modern farming practices and found that it

contributed between 3% and 72% to yield depending on the soil type and rainfall distribution of the site.

In addition to evidence from direct experiments and modelling in wheat, empirical studies with different crops demonstrate the value of deep roots to yield under drought in the field. Root depth has been positively correlated with yield in soybean (Cortes and Sinclair, 1986). In rice, benefits have been shown to accrue from increased root depth even under water stress (Kamoshita *et al.*, 2002). Also in rice, maximum root length, root depth, and basal thickness were correlated with yield in drought conditions (Champoux *et al.*, 1995; Li *et al.*, 2005). In a study linking traits in the laboratory with yield under drought in the field, Li *et al.* (2005) showed a positive correlation between root depth and yield in rice.

Root system traits to increase uptake of stored soil moisture

Traits to increase root system depth and deeper water uptake are explored below. We cover shoot traits that may indirectly impact on root traits that access more deep water, and root traits that could directly increase water uptake from depth.

Trait 1. Deeper root systems (see Fig. 1)

One effective approach to increase root depth has been to increase the time to reach flowering. The duration of root descent in wheat is approximately related to the duration from sowing to flowering as downward growth ceases around the time of flowering and onset of grain development (Gregory *et al.*, 1978). Time to flowering has been manipulated in Australian breeding programmes by sowing earlier in the season with varieties containing vernalization and photoperiod genes that extend the pre-flowering period (Richards, 2006). Earlier sowing improves water-use efficiency and root depth in Australian studies (Gomez-Macpherson and Richards, 1995; Kirkegaard *et al.*, 2007). Precise knowledge of wheat vernalization and photoperiod genes allows for marker-assisted breeding to adjust flowering times and duration of vegetative growth to maximize root depth and capture of soil water in a specific region and at a specific sowing time (Eagles *et al.*, 2011). Variation in post-anthesis root growth can also have significant effects on grain yield. Important differences in the spatial distribution of post-anthesis root growth have been found between wheat genotypes (Manschadi *et al.*, 2006). Increasing total post-anthesis root growth would require allocation of more carbon to roots post-flowering that could come from more green leaf area, as is possibly the case in the wheat cultivar Seri (Manschadi *et al.*, 2006; Christopher *et al.*, 2008), or from increased specific photosynthetic capacity. Alternatively, it could be possible to increase root length density at depth without extra carbon input by modifying specific root length.

An increase in root system depth may result from a faster rate of root system elongation, also referred to as 'root

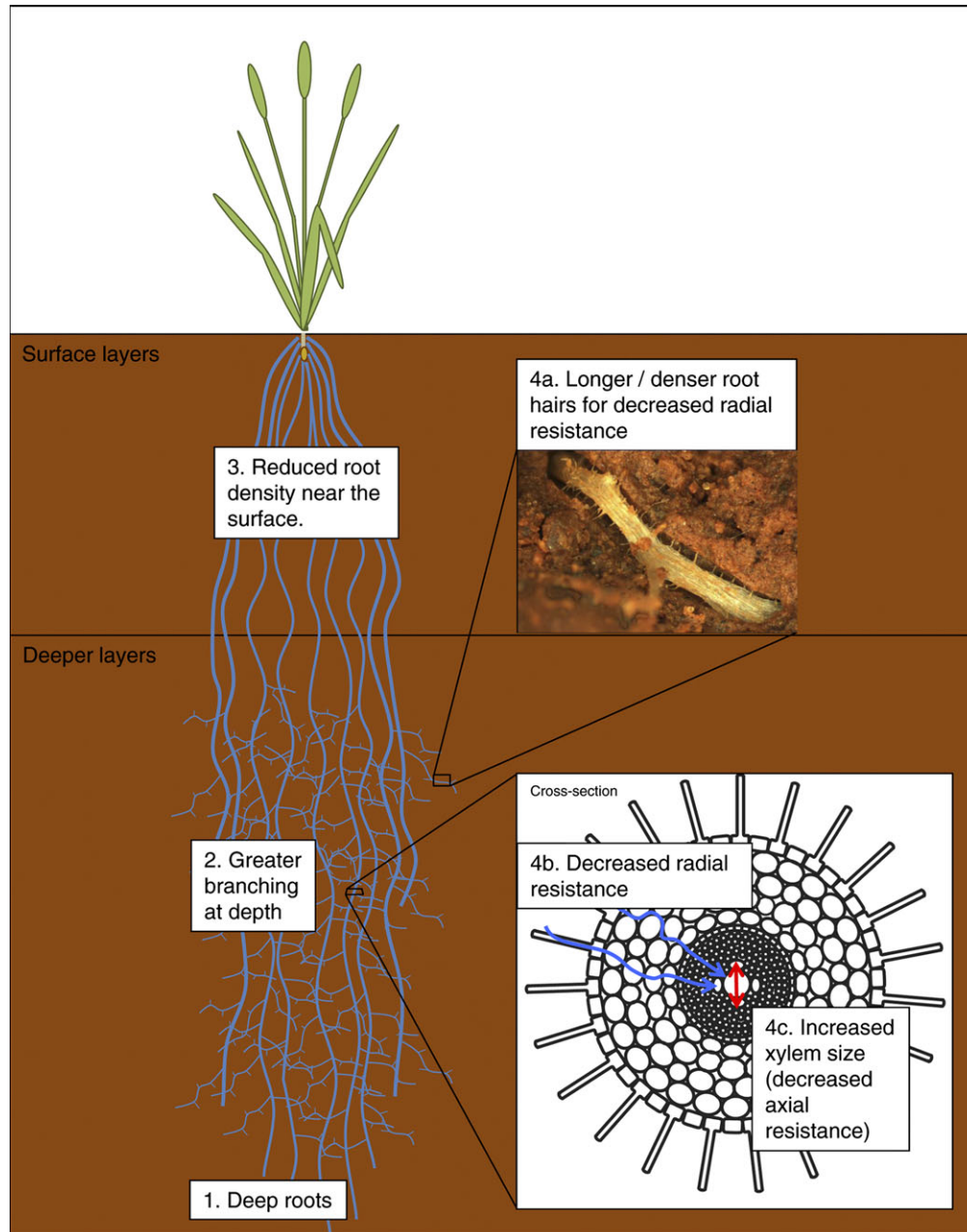


Fig. 1. Diagram illustrating four traits to increase deeper water uptake. (1) Deep roots to increase the amount of subsoil moisture which the root system could access. (2) Greater root length density to enable more complete uptake of the soil moisture. (3) Reduced root length in surface soil. (4) Reduced resistance to water movement from soil to shoot by longer and denser root hairs (4a), decreased radial resistance to water movement in roots (4b), and increased xylem size to decrease axial resistance to water transport to the shoot (4c). Figure 4a kindly provided by Rosemary White, CSIRO.

vigour' (Palta and Watt, 2009), and/or a narrower angle of descent (discussed below). There are different terms used in the literature, such as 'root system descent rate', 'root front velocity', and 'penetration rate'; 'root vigour' herein refers to the overall rate of root system elongation, whereas 'descent rate' refers specifically to the rate at which maximum root depth increases. Faster root growth depends on processes within the root apex that determine cell division and expansion (Sharp *et al.*, 2004). Genotypes with these features may be selected in screens that directly measure the root elongation rate. Root vigour also depends

on photoassimilate and water allocation to root tips for growth (Boyer *et al.*, 2010), suggesting that manipulation of shoot growth would provide extra resources to root growth. In another example of using shoot traits to manipulate root traits, selection programmes for high shoot vigour have resulted in lines with more vigorous early root growth (Watt *et al.*, 2005; Liao *et al.*, 2006). This early root vigour in small grain cereals may be associated with a deeper root system in the field (Richards, 1991; Richards *et al.*, 2007). It is also possible to use shoot tiller number (branch number) to increase root vigour. In rice and wheat, it is

observed that genotypes with fewer tillers have deeper root systems (Yoshida *et al.*, 1982) or longer root systems (Duggan *et al.*, 2005; Richards *et al.*, 2007).

The angle at which roots penetrate the soil may also relate to root depth. Root angle has been selected in bean breeding programmes and has resulted in varieties with shallow root systems to access phosphorus in the surface soil (Liao *et al.*, 2001; Lynch, 2011; Lynch and Brown, 2001). The angle at which roots emerge from the seed could be used as a proxy for deep rooting characteristics, particularly if it reflects an underlying gravitropic tendency in the root system. The gravitropic tendency could increase the depth as more root growth is directed to descent and not spread. Modelling suggests that selection for a narrow angle in wheat root systems results in deeper root growth and higher yields (Manschadi *et al.*, 2008). In seedling screens of 26 Australian wheats, those adapted to drier environments where water at depth is frequent tended to have the narrowest horizontal root spread, while those adapted to Mediterranean conditions where most water for the crop comes from frequent but low rainfall events during the season tended to have a wider spread (Manschadi *et al.*, 2008). However, in another study, no correlation between wider root angle in seedling screens and higher yield was found in Australian Mediterranean environments (McDonald, 2010). The root growth angle of Japanese winter wheat varieties, assessed in controlled environments, was shown to correlate negatively with the vertical root distribution of those varieties in the field (Oyanagi and Nakamoto, 1993). Wild barley, which evolved in water-limited conditions, has a narrow angular spread when compared with modern cultivars (Bengough *et al.*, 2004). In summary, evidence suggests that the angle of early root formation may determine rooting depth. However, proof of concept in the field is required in populations where divergent selection has been practised for root angle, especially given the strong influence of soil structure on root growth and distribution at depth (White and Kirkegaard, 2010).

The root tip drives the gravitropic response in an auxin-dependent mechanism that alters cell elongation. Many components of the auxin transport pathway are involved (Swarup and Bennett, 2009). It is less obvious whether the mechanism for a gravitropic tendency may drive other root traits, such as increased growth. The rice OsIAA31 mutant (Nakamura *et al.*, 2006) has defects in gravitropism and root length, suggesting overlap in the interpretation of the auxin signal in cell elongation. A strong gravitropic response may be a marker of the underlying hormonal control of root depth. The gravitropic response of wheat seminal roots was shown to be heritable, with the results suggesting it was under the control of a single dominant gene (Oyanagi *et al.*, 1991).

Methodologies for the selection of root growth angle in seedlings have included, in order of increasing sophistication, germination paper pouches (Liao *et al.*, 2001), semi-hydroponics (Chen *et al.*, 2011), gel-filled chambers (Bengough *et al.*, 2004), and three-dimensional gel-based imaging setups (Iyer-Pascuzzi *et al.*, 2010; Clark *et al.*, 2011).

Trait 2. Increased root length density in medium and deep soil layers (Fig. 1)

It is estimated that ~1 cm of root is required to extract the plant-available water from 1 cm³ of soil, with sufficient time, root-soil contact, and hydraulic conductance in the root (Passioura, 1983). At harvest, crops with insufficient root length density at depth can leave water behind in deeper soil layers (Li *et al.*, 2002). Using modelling and historic rainfall data, Lilley and Kirkegaard (2011) showed that a faster root descent rate (20%) resulting in deeper roots, coupled with more efficient subsoil water extraction (20% beneath 0.6 m depth), would produce mean yield benefits of 0.32 t ha⁻¹ in southern Australian sites which have water from in-season and stored rainfall, and 0.44 t ha⁻¹ in northern Australian sites, which rely mainly on rainfall stored in the soil from pre-season summer rains. Direct quantification of the bottom third of wheat, barley, and triticale root systems in the field showed that 6% of the roots were axile roots; the remainder are branch roots (Watt *et al.*, 2008). Where increased depth increases the volume of soil moisture available for capture, more length from branches is needed to improve the capture of that water. Increased branch length arises from more and longer branch roots caused by the initiation and development of branch root primordia in the pericycle; faster elongation rates of those branch roots; and delayed onset of dormancy of the branch root meristems. Assimilate allocation is presumably the primary determinant of elongation rates and vigour, as discussed above; whereas the initiation of branch root development and cessation of elongation are likely to be controlled by other mechanisms, such as plant hormones.

Hurd (1964) was the first to select directly for root density in a breeding programme. He studied patterns of root development in root boxes with clear faces, to identify a cultivar that displayed this trait under a variety of soil moisture conditions. He then demonstrated its high yields in dry seasons in the field (Hurd, 1964).

A number of empirical studies support Hurd's breeding efforts for increased root density. Sorghum varieties from suboptimal environments have a greater degree of branching (Masi and Maranville, 1998). Soybean cultivars with greater root density appeared to have increased water uptake, and soybean cultivars with extensive fibrous root systems in the surface layers appeared to be more drought tolerant (Carter *et al.*, 1999; Pantalone *et al.*, 1999). In upland rice, root depth, density, and number were all positively correlated with water acquisition (Price *et al.*, 2002). Even in shallow soils, an emphasis on root length density in the 'deeper' layers can contribute to improved water uptake. In rice, varieties with increased root length density in the 35–45 cm layer had improved resistance to drought (Henry *et al.*, 2011).

However, the value of greater root length density to yield in dry conditions is variable. In a study of modern and older wheat varieties in the sand over clay soils of Western Australia, Siddique *et al.* (1990) showed that the older varieties had more root dry matter and root length density in the top 40 cm of the soil profile, but there was no

variation for water extraction or water use. They attributed higher harvest index and water-use efficiency in the grain amongst modern varieties to a reduction in root dry matter and a decrease in the root to shoot ratio. However, this study was also confounded with variation in the duration of the pre-flowering period. Palta *et al.* (2011) recently concluded that root systems with more total root length were of value in climates that received rain through the season, but may exhaust stored soil moisture where it was the sole source of available water, and shoot vigour was associated with high root vigour.

Trait 3. Reduced root length density in the topsoil (Fig. 1)

In most wheat crops, root length density in the surface layers is 3–5 cm cm⁻³ and thus, in theory, exceeds that required to extract crop available water (Passioura, 1983). Rainfed wheat crops, where limited rainfall is expected in the latter part of the season, may benefit from a reduction in topsoil roots. This excess root length may be a carbon cost to grain, especially if grown towards the end of the season when carbon is limiting, and rainfall events are short and evaporate rapidly before root water uptake. On the other hand, this extra root length density may be critical for nutrient capture.

A study in maize suggested that higher density of roots in the drying soil layers may also be associated with an increased flux of abscisic acid (ABA) towards the leaves, retarding transpiration and grain setting (Giuliani *et al.*, 2005). Similarly, a split-root experiment in rice with flooded and drought-treated roots showed that stomatal conductance and transpiration were reduced before there was a reduction in leaf water potential, an early drought response associated with increased ABA. Severing the drought-treated roots led to a recovery in leaf water potential, suggesting a role for hydraulic signalling mediated by the reduction of hydraulic conductivity through cavitation (Siopongco *et al.*, 2008). The strength of this response varies genotypically, with an upland (dry) line showing reduced ABA signalling and superior recovery to a lowland (flooded) line (Siopongco *et al.*, 2009). A study in barley showed that, independent of the amount of water available to the plant root system, varieties with fewer roots in drying soil had more leaf growth and less foliar ABA than those with more roots in drying soil (Martin-Vertedor and Dodd, 2011). However, foliar ABA may not be the appropriate measure of this signalling, as pearl millet near isogenic lines that differed in foliar ABA concentration were not shown to differ in total water extraction (Kholova *et al.*, 2010). This root to shoot signalling could be the focus of breeding efforts to reduce sensitivity.

Roots in the topsoil of older wheat plants are primarily nodal axile roots and their branches. It may be possible to redirect them to deeper layers, perhaps by selecting for narrower angles of growth. Alternatively, selection could be made against excessive nodal root growth. Abolishing nodal roots would also reduce associated root length density by branch roots (Oyanagi *et al.*, 1993, and references therein).

Nodal root formation is under the control of a single gene in maize, *WOX11*, a transcription factor that is auxin and cytokinin inducible (Zhao *et al.*, 2009). However, a cautious approach should be taken to the extreme manipulation of nodal roots. The hormonal evidence suggests overlapping pathways for nodal and branch root development. In *Arabidopsis*, WOX-like genes have been shown to play a role in lateral root initiation, which suggests overlap in the control pathways for these two types of roots (Deveaux *et al.*, 2008). Both branch and nodal roots have been shown to be generated as part of plastic responses to environmental cues. This suggests that alterations in nodal root development may have unwanted side effects in branching in seminal and other earlier nodal axile roots, which may impede our attempts to improve root density at depth. Another detrimental side effect of reducing roots in the topsoil is reducing nutrient uptake, for example immobile P or Zn, which is valuable when taken up during grain development. It also increases the chance of severe root system restriction if a main axis is rotted by a pathogen such as *Rhizoctonia* fungi.

Surface roots that respond to frequent small rainfall events with additional growth may be a valuable trait in Mediterranean environments (Sadras and Rodriguez, 2007). The moist soil surface may dry before new roots become functional, but repeated showers may be captured by an expanded surface root system. Given the cyclical wetting and drying that occurs in surface layers, shoot characteristics that result in more shading of the soil surface, reduce evaporation, and maintain saturation of the air are likely to be of value to the growth and function of surface roots (Rostamza *et al.*, unpublished results). These shoot traits may and could be more easily selected in the field than root traits.

Trait 4. Decreased resistance to water movement from soil to root by increasing root hair growth and xylem diameters (Fig. 1)

Two types of resistance determine the uptake of soil water by roots: radial resistance, the resistance of water passing from the soil into the root and to the vasculature; and axial resistance, the resistance of water passing from the root to the shoot through the vasculature. The relative contribution of radial and axial resistances to incomplete soil water uptake was studied by Rowse and Goodman (1981) who concluded that radial resistance was a greater determinant of water uptake than axial resistance.

Richards and Passioura (1989) engaged in the best known example of the manipulation of a developmental root trait in wheat; reducing the xylem diameter of seminal axile roots to increase their axial resistance to water from the root system to the shoot, so that soil water uptake earlier in the season was reduced, leaving soil moisture available during grain filling where it contributed directly to the harvest index. Yields were improved in years of water stress, but the reduced xylem diameter did not adversely affect yields when there was abundant rainfall, as the nodal roots developed to

exploit the additional rainfall (Richards and Passioura, 1989). By combining high axial resistance at the base of the root system with lower axial resistance in deeper roots, it may be possible to delay use of soil water until flowering and grain development to increase the harvest index, and exploit more fully water from deeper soil layers.

Incomplete utilization of subsoil water has been attributed to resistance at the soil–root interface (Passioura, 1991). Root hairs provide a mechanism by which the plant roots' contact with the soil can be maximized and the resistance minimized (Fig. 1). Segal *et al.* (2008) analysed water uptake by barley mutants lacking root hairs and showed that, despite greater branching, the bald roots had less water uptake and less uptake per unit root length. White and Kirkegaard (2010) observed and quantified the location and root–soil contact of deep wheat roots in the field in an Australian clay soil. They did not grow through soil uniformly, but instead exploited pores and channels and contacted the soil through root hairs. Root hairs are especially noticeable where there is a gap between the root surface and the soil (Fig. 1), and clearly provide hydraulic continuity between root and soil. Direct selection for longer and denser root hairs, or hair development that contacts soil in gaps and pores, using imaging would be challenging given the high variability of root hairs in the field, and the possibility that seedling root hairs in the laboratory are not the same as hairs on components of mature root systems in field conditions. Two genes for root hair elongation, *RTH1* and *RTH3*, have been identified in maize, and may be valuable for genetic improvement (Hochholdinger and Tuberosa, 2009).

Improving uptake by lowering axial resistance has been proposed for rice. Nguyen *et al.* (1997) proposed that increasing xylem size and lowering axial resistance would allow for better exploitation of water in deeper soil layers. This was supported by studies linking root thickness with drought resistance (Ekanayake *et al.*, 1985) and quantitative trait loci (QTLs) for basal root thickness with yield in dry upland, but not wet lowland, cultivation (Champoux *et al.*, 1995; Li *et al.*, 2005). The correlation of root thickness and xylem size has been made in rice, but the conclusion that xylem size contributes to drought resistance could not be drawn (Yambao *et al.*, 1992).

Applicability of the traits

The traits discussed are likely to be of value in a rainfed wheat production system with summer-dominant rainfall and evidence of stored soil moisture, particularly at depth. However, there may be situations where these traits may not be beneficial.

The deep root trait and the dense root at depth trait are likely to be valuable where there is a shortfall of water late in the growing season, a common occurrence globally. As discussed earlier, in Mediterranean environments with frequent in-crop rainfall events, there may be little or no benefit to deep root systems and they may pose an additional burden on plant development (Palta *et al.*,

2011). Similarly, in situations where there is deep drainage, there may be little stored soil moisture to access.

In environments with particularly shallow soils, deeper root systems will not be of benefit, and focus should instead be on better capture of rainfall events (where available), traits to minimize the impact that water deficit has on plant transpiration, and traits to meter out the available water over the entire season (such as reducing xylem size).

Reducing root length density in the topsoil may be particularly inappropriate in Mediterranean environments, where shallow roots may increase the capture of in-season rainfall events.

High-throughput techniques for the direct evaluation of root systems in the field do not yet exist

Identifying desirable root phenotypes directly in the field would be the shortest route to the incorporation of traits of value in a crop-breeding programme. However, that approach is blocked by the lack of high-throughput phenotyping techniques for the field.

Traditional studies have focused on excavation techniques, from which root depth and root length density can be determined. Trenching is labour intensive and slow (Van Noordwijk *et al.*, 2000). Many comparisons between varieties can be made in a day using mechanised soil coring with core-break counts, which correlate well with washed root length densities (Drew and Saker, 1980; Bennie *et al.*, 1987). Core sample processing has also been improved with automatic washing systems (Smucker *et al.*, 1982; Pallant *et al.*, 1993), imaging of washed roots with flatbed scanners, and software packages for analysing the washed images (French *et al.*, 2009; Le Bot *et al.*, 2010; Lobet *et al.*, 2011).

Minirhizotrons are a non-destructive alternative to excavation techniques, where a transparent tube is inserted into the ground and root growth abutting the tube is imaged with a camera that is inserted down the tube (Smit *et al.*, 2000). Because minirhizotrons are non-destructive, the operator may monitor root growth and turnover. However, the roots must first grow against the tube wall, limiting what can be analysed. Furthermore, the interface of the tube and soil is an artificial environment for root growth, which may lead to incorrect assessments of the growth characteristics of the plant. Minirhizotrons have been shown to over- and underestimate root length density depending on the species examined and installation angle (Bragg *et al.*, 1983; Heeraman and Juma, 1993; Rytter and Rytter, 2011; Vamerli *et al.*, 2012).

Ground-penetrating radar has been explored as a technique for root measurement, but resolution limitations mean that it is likely to be restricted to trees and woody plants (Zenone *et al.*, 2008). Electrical resistance tomography has been applied to mapping soil physical properties in agricultural systems (Basso *et al.*, 2010) and the exploitation of soil water by roots in a crop stand (Srayeddin and Doussan, 2009)—both of which are indirect measures of

root behaviour. However, electrical resistance, measured with electrical resistance tomography (ERT), has been correlated with soil moisture and root biomass for alfalfa growing in a small container (Amato *et al.*, 2009)—offering the possibility that it could be used for the *in situ* detection of roots in the field.

Electrical capacitance measures of root system size were pioneered in the late 1970s (Chloupek, 1972, 1977), and a theoretical basis has been developed (Dalton, 1995). Capacitance was correlated with root system size in maize at flowering in the field; however, effective measurement is strongly dependent on the soil being moist when the measurements are performed (van Beem *et al.*, 1998). It has also been employed in the study of tree roots (Ellis *et al.*, 2008).

In the authors' experience ~50 genotypes, replicated four times, could be soil cored with a tractor-mounted hydraulic push press and four people in one day. The distribution of mature root systems was assessed in 10 cm increments to a depth of 2 m using core-break counting. Correlation with a subset of samples that were washed and scanned (~3 d) was ~0.75. Thus, until non-destructive methods are validated and made to be more rapid, root distribution and rooting depth can be reliably assessed rapidly at 2–3 min per core in the field with a traditional technique such as soil coring.

Laboratory and field proxy screens and their pitfalls

Trait-based selection programmes for breeding require selection methods that are high throughput for high numbers of lines, and that correlate with yield. As discussed, techniques for rapid direct root phenotyping in the field are not available, particularly for mature, deep root systems. Thus, high-throughput screens in the laboratory or the field for a 'proxy' trait must be used. A 'proxy' trait is a shoot or root measurement that is likely to be a consequence of, or correlate with, the desired phenotype. For example, root angle can be thought of as a proxy for deeper roots in the field, as can high shoot vigour.

The four traits highlighted above can be selected by proxy in the laboratory at the seedling stage. However, deeper soil water is captured by adult plant root systems. Mature root system traits, such as root depth and increased branching at depth, are highly dependent on soil and seasonal climatic factors, and on plant phenological stage. Seedling roots in the laboratory may not express the developmental features of mature roots. It is assumed that the genetics that drive the expression of the proxy trait in the laboratory also drive the expression of the desired trait in the field, and that this explains the correlation. This is the major pitfall of laboratory screens; they rely on measurements in young plants that may not confer the desired phenotype in the field at maturity.

An alternative to laboratory screens is to use shoot proxy measurements in the field to identify varieties with valuable

root systems. The simplest of these is to assess shoot performance such as shoot biomass, yield, grain weight, maintenance of green leaf area, etc. Other measurements could include canopy temperature (CT) measured with an infrared thermometer or camera allowing selection of genotypes in the field with cool canopies. A cool canopy indicates transpiring leaf area and can be an indirect measure of crop access to water by the root systems, another proxy trait (Blum *et al.*, 1982, 1989; Garrity and O'Toole, 1995). CT was used for the remote sensing of wheat lines with deeper roots, and CT at grain filling negatively correlated to root dry weight at depth (Lopes and Reynolds, 2010). However, numerous factors independent of root depth may affect CT in a segregating population. Ignoring these has led to confusion among breeders on the utility of screening for CT in breeding populations. These factors (including biomass, canopy height, and development) must be considered when selecting for genotypes producing cooler canopies (Rebetzke *et al.*, 2012). The isotopic signature of carbon in the grain may also be a valuable indicator of differences in access to water by mature root systems (Araus *et al.*, 2003). Genotypes with roots that access more deep water and maintain photosynthetic leaf area and stomatal conductance during grain filling will generate grain with a different ratio of ^{13}C to ^{12}C from those that rely on carbon fixed earlier in the season or carbon fixed by a plant under drought stress and with a low stomatal conductance. However, as for CT above, the use of grain $^{12}\text{C}/^{13}\text{C}$ discrimination relies on good understanding of tested populations and assumes no confounding of genotypic differences in anthesis biomass, pre-anthesis water use, and remobilization of stem carbohydrates (Condon *et al.*, 2002; Rebetzke *et al.*, 2008).

The major pitfall of indirect field proxy screens is that the shoot measurements or soil water measurements can be greatly influenced by the field site and weather in the year of testing. Imagine a field screen for root systems (depicted in Fig. 2) where yield measurements are used as an indirect screen for root depth (not dissimilar from the assumption that yield breeding produces the variety with the optimal root system). The plant on the right has a shallow root system, but one that is fully functional. It has limited capacity for water uptake (at depth) and delivers the lowest yield (depicted here as a single head). The plant in the middle has an improved root system, which is fully functional. This is the highest yielding variety; having an optimized root system it has the best water uptake for the investment in the root system. The variety on the left is the most unusual; a genetic alteration has caused its roots to develop with exceptional depth and density. This plant possesses a trait that we imagine an 'ideal' plant could possess. However, some other physiological constraint (perhaps insufficient hydraulic demand in the shoots, or insufficient xylem capacity) renders this deeper root system non-functional. The variety is, in our hypothetical system, only capable of taking up the same amount of water as the variety in the middle, but the energetic demands of its larger root system result in a lower yield in our screen.

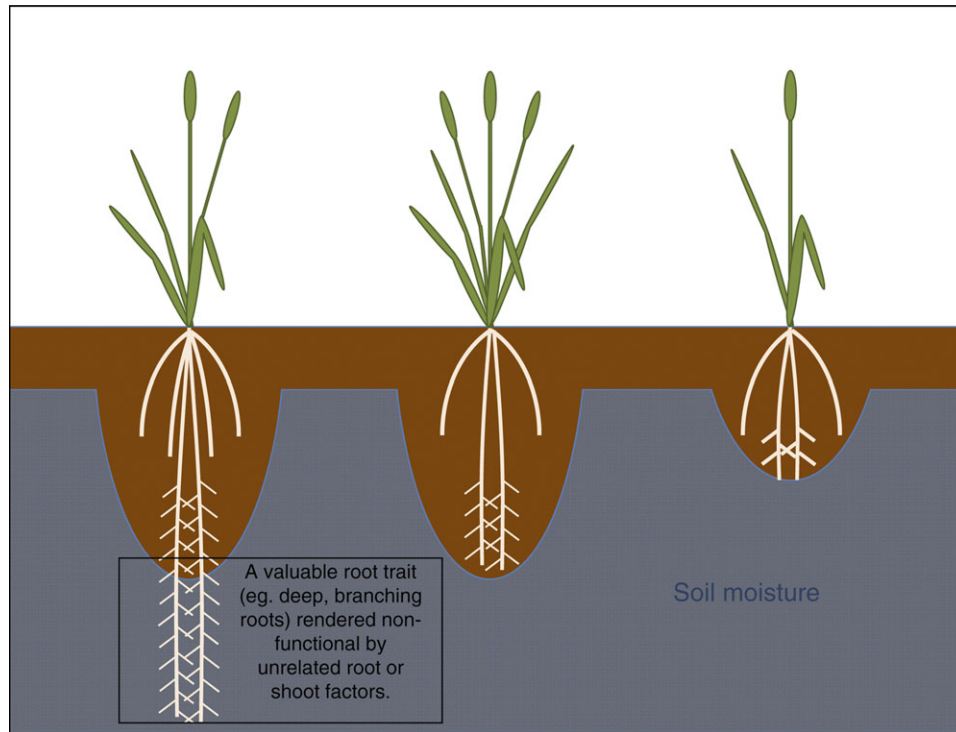


Fig. 2. Cartoon illustrating the problems of reliance on indirect measures of root growth. The plant on the left has the best root traits (here imagined to be deep, highly branched roots), but an unrelated root or shoot constraint negates the advantage. The non-functional roots are an energetic burden on the plant. Hence, in an indirect screen, the plant on the left will not perform as well as the plant in the middle, which has a completely functional root system, even though it does not have the superior root trait (the deep highly branched roots). The middle plant will, however, out-perform the plant on the right on the basis of superior, functional, root traits.

Some may argue that the middle variety has the superior genetics, because its root system is optimized. However, the alternative view is that the variety on the left has the superior root genetics. Why? Because, superior root systems may not confer superior yields upon a variety where a physiological constraint outside of the root system limits the root system's contribution to yield.

Thus, the use of indirect screening within trait breeding should be considered with caution. Trait breeding is based upon the identification of lines that possess that trait ideal. However, if the ideal does not have a functional impact on the proxy trait, then ideotype identification will not occur. The value of the trait is in the genetics that confer the trait and the particular environment and year in which it is being tested.

A selection programme with laboratory and field measurements at the start to identify a worthwhile proxy screen and develop varieties to increase deep water uptake and yield

Here we propose a programme with laboratory and field measurements in parallel in search of a worthwhile proxy at the outset of the trait-breeding programme (Fig. 3), to

establish a screen that confers deeper roots with greater water uptake and yield, and speed up development of a new variety. This ensures that large investment is not made in a screen that does not confer a field advantage. Ideally, a laboratory screen could be identified. This would be much quicker and more reliable than the field proxy screen as multiple generations could be advanced annually. The parallel laboratory and field measurements at the outset greatly help to build confidence in a laboratory proxy screen, which, as mentioned above, generally relies on a seedling trait that may not necessarily translate to mature root system depth and deep water uptake.

The basis of this programme is the definition of a specific target environment, the constraints in those environments, and the rational selection of trait combinations that are likely to overcome any specific constraints (see section 'Applicability of traits'). This potentially reduces any genotype \times environment ($G \times E$) interaction that may limit selection efficiency.

Critical in this programme is the selection of the appropriate field environments and management practices, to correlate the proxy trait with deep roots, deep water uptake, and yield. The expression of the trait is expected to be greatly influenced by the specific field conditions encountered and so management of these proxies and interpretation of the response is very important. Field site knowledge, weather measurement, and consistent management practices ensure

that deep water is present, and help to stabilize the field environment year to year and to avoid the pitfalls highlighted in Fig. 2.

At the outset, laboratory and field proxy traits for the ideotype root trait are conceptually identified (Fig. 3). These are developed and evaluated between the laboratory and the field, looking for high repeatability, low G×E, high heritability, and cost-effectiveness (high numbers of lines with low growing and measurement costs). Most importantly, any laboratory proxy screens must be expressed in the field through to maturity and confer the desired trait, for example deeper roots with greater deep water uptake. Once the effective screen is developed, germplasm is screened to identify a donor of the trait. This may be from a diverse, global selection, biased with old and current high-yielding lines from environments with deep water to maximize diversity in the proxy. Alternatively, there may be a single genotype as a source of the trait already identified from field evidence and controlled environment studies, such as in the case of Seri, which was intensively studied in large root boxes (Manschadi *et al.*, 2006). The best germplasm to start with, provided it has wide diversity for the proxy trait, is a mapping population because the trait can be linked to a genomic region for marker development. Also mapping populations are comprised of sister lines and will therefore share a common ancestry. This reduces the risk of the pitfalls of Fig. 2, where field expression of the proxy can be masked by genetic differences in processes that have a large effect on water use such as disease resistance and flowering time. The genes from the

donor parent of the trait are progressed through a backcross programme to develop a commercial cultivar with the trait and other desirable characteristics for yield, including disease resistances and quality (Fig. 4). In this scheme, progeny are enriched for the trait with the screen or a marker for the trait, and those that contrast for expression of the proxy trait (e.g. ‘tails’) can be evaluated in the field, to continue to build confidence in the trait. This is done from the outset, and year to year as the sister lines become more genetically similar. This ensures that the proxy correlates adequately with the desirable trait. If the proxy trait fails at any stage it can be abandoned before further costs are incurred. It also ensures that diversity in the ideotype is being incorporated in the selection of the source trait, and that the ideotype trait is being inherited through the breeding programme for the proxy trait. It also allows an early evaluation of the contribution of the trait to performance, and the identification of any constraints on the translation of the trait to performance. The approach should more rapidly lead to material with valuable root traits, especially if the pitfalls of proxy screening discussed above are minimized.

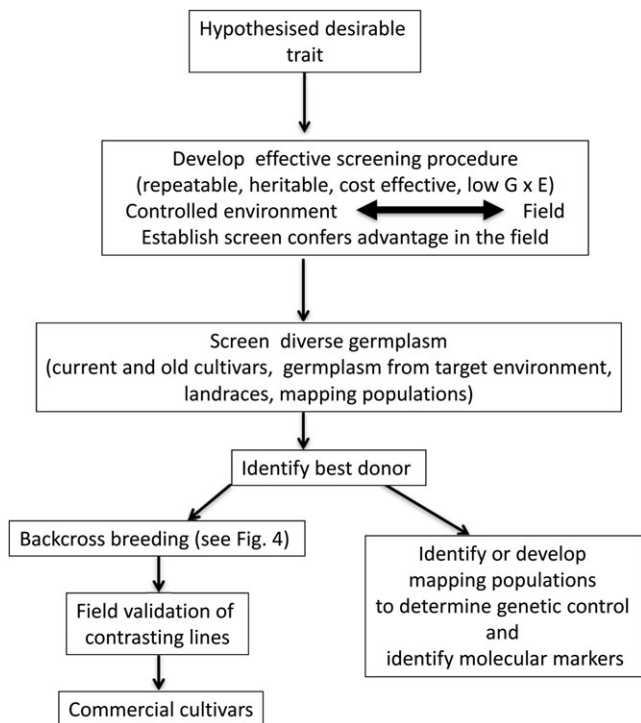


Fig. 3. A selection programme with laboratory and field measurements at the start to identify a worthwhile proxy screen and develop varieties to increase deep water uptake and yield.

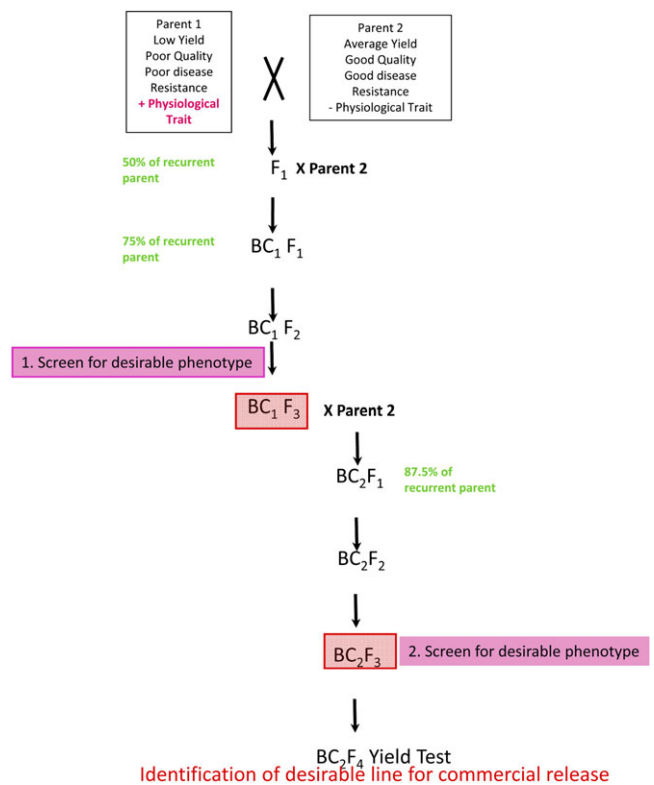


Fig. 4. Backcross breeding scheme for selection of germplasm with a desirable phenotype. This scheme is followed after an effective screen for the trait has been identified and the best donor of the trait has been identified (see Fig. 3). The scheme allows incorporation of desirable yield traits from Parent 2 at the initial cross, enrichment of lines carrying the desirable phenotype with the phenotypic screen or marker, validation of the trait value in the field at (1) and (2), and identification of a desirable line for commercial release.

Conclusion

A review of the evidence has shown the considerable value of stored soil moisture to wheat crops in many environments and suggests an ideotype breeding approach to optimize exploitation of this resource. Four traits have been proposed to improve productivity where this resource is available: deeper root systems, increased root density at depth, decreased root density at the surface, and decreased resistance to the movement of water from soil to roots through an increase in root hairs and/or xylem diameters. None of the field-based methodologies reviewed is sufficiently high throughput for a root-breeding programme. Indirect 'proxy' traits are argued to be problematic in an ideotype breeding approach, so a programme of repeated revalidation of these traits against direct, low-throughput, measurements in the field is advocated. This approach can be utilized in other breeding programmes. The key steps are the identification of the root-based constraints on productivity in a specific target environment, the identification of ideotype traits to overcome this constraint, and a breeding programme, continuously validated in the target environment. Laboratory and field research 'talk to each other' from the outset.

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