

REVIEW PAPER

Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement

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Received 18 November 2013; Revised 10 March 2014; Accepted 11 March 2014

Abstract

Several root anatomical phenes affect water acquisition from drying soil, and may therefore have utility in breeding more drought-tolerant crops. Anatomical phenes that reduce the metabolic cost of the root cortex ('cortical burden') improve soil exploration and therefore water acquisition from drying soil. The best evidence for this is for root cortical aerenchyma; cortical cell file number and cortical senescence may also be useful in this context. Variation in the number and diameter of xylem vessels strongly affects axial water conductance. Reduced axial conductance may be useful in conserving soil water so that a crop may complete its life cycle under terminal drought. Variation in the suberization and lignification of the endodermis and exodermis affects radial water conductance, and may therefore be important in reducing water loss from mature roots into dry soil. Rhizosheaths may protect the water status of young root tissue. Root hairs and larger diameter root tips improve root penetration of hard, drying soil. Many of these phenes show substantial genotypic variation. The utility of these phenes for water acquisition has only rarely been validated, and may have strong interactions with the spatiotemporal dynamics of soil water availability, and with root architecture and other aspects of the root phenotype. This complexity calls for structural-functional plant modelling and 3D imaging methods. Root anatomical phenes represent a promising yet underexplored and untapped source of crop breeding targets.

Key words: Aerenchyma, cereals, cortex, drought, root anatomy, xylem.

Introduction

Drought is a primary constraint to agriculture and continues to have significant impacts in both developed and developing countries. This problem will be exacerbated by climate change, resulting in increased incidence and severity of drought in many regions, and consequently reduced food production (Lobell and Gourdj, 2012). The human population is projected to reach 9.6 billion by 2050 (Lee, 2011), placing even greater pressure on world food security, especially in developing countries where >80% of the population increase is expected to occur (Roberts, 2011). The development of crop cultivars with enhanced drought adaptation and higher yield has been the focus of many crop improvement programmes. Roots play key roles in water acquisition and are a significant component of plant adaptation and fitness in water-limited

environments. New crop cultivars with improved root traits for water acquisition from drying soil could have a significant impact on global food security.

Genotypic variation for root traits and their functional implications for water acquisition and increased yields under water-limited conditions have been reported for many crops. Such variation includes differences in anatomical phenes that can reduce the metabolic cost of soil exploration (Zhu *et al.*, 2010a; Burton *et al.*, 2013; Jaramillo *et al.*, 2013) and root architectural phenes capable of optimizing soil exploration in time and space (Lynch, 1995, 2013; Manschadi *et al.*, 2006; Henry *et al.*, 2011, 2012). In this review we concentrate on root anatomical traits that enhance water acquisition from drying soils. We will first discuss anatomical traits that can

improve water acquisition from drying soils by reducing the metabolic costs of soil exploration, followed by anatomical traits that influence radial and axial water transport. We then consider traits that improve root penetration of hard, drying soil. Finally, the prospects of deploying these traits in crop improvement will be discussed.

Traits that reduce the metabolic cost of soil exploration

A number of studies have shown that the metabolic costs of soil exploration by root systems are substantial, and can exceed 50% of daily photosynthesis (Lambers *et al.*, 2002). Following the economic paradigm of plant resource allocation (Bloom *et al.*, 1985), we use the term ‘cost’ to denote metabolic investment, including the production and maintenance of tissues, often measurable in units of carbon (Lynch and Ho, 2005). All else being equal, a plant that is able to acquire a limiting soil resource at reduced metabolic cost will have superior productivity, because it will have more metabolic resources available for further resource acquisition, growth, and reproduction.

The importance of root costs in soil resource acquisition is illustrated by the case of phosphorus (P) acquisition in common bean (*Phaseolus vulgaris*). In bean, low P availability increases the fraction of daily photosynthate respired by roots by 75% in both P-efficient and P-inefficient genotypes (Nielsen *et al.*, 1998, 2001). However, P-efficient genotypes have greater root growth per unit root respiration than P-inefficient genotypes (Nielsen *et al.*, 2001), which enables P-efficient genotypes to develop more than twice as much root biomass at low P than the P-inefficient genotypes. P stress slightly increases the respiration per unit biomass of roots of the P-inefficient genotype, but halves the respiration of roots of the P-efficient genotype (Lynch and Ho, 2005). Thus, adaptation to low P availability in bean is associated with the ability to explore the soil at minimal metabolic cost (Lynch and Ho, 2005; Lynch and Brown, 2006). The importance of root costs for soil resource acquisition was supported by a study with the functional–structural plant model SimRoot, which showed that root maintenance respiration accounts for a substantial portion of the effects of nitrogen (N), P, or potassium (K) deficiency on the growth of maize plants (Postma and Lynch, 2011). The model predicts that under severe N or P deficiency, root maintenance costs can reduce the growth of 40-day-old maize plants by almost 40%, and under severe K deficiency, by >70% (Fig. 1). These studies in controlled environments and *in silico* underestimate actual root costs, which under field conditions include root loss to abiotic and biotic stress (Fisher *et al.*, 2002).

Less is known about the root costs of crops under water stress, but it is known that water stress substantially increases root growth relative to shoot growth (Eghball and Maranville, 1993; Palta and Gregory, 1997). A greater root to shoot ratio means that each unit of leaf area has more non-photosynthetic tissue to sustain, which reduces the overall plant growth rate (Hunt, 1982; Poorter and Remkes, 1990). As demonstrated

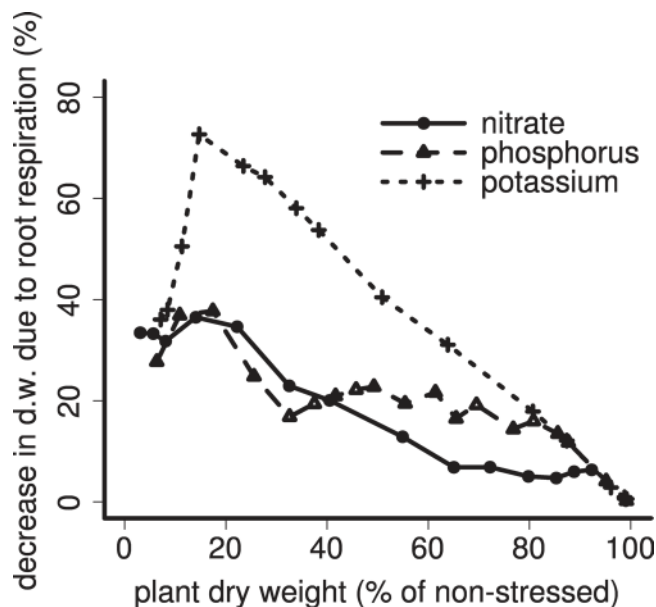


Fig. 1. Growth reduction [percentage plant dry weight (d.w.)] due to root maintenance respiration in 40-day-old maize plants subject to varying degrees of deficiency of N, P, and K. From Postma JA, Lynch JP. 2011. Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiology* 156, 1190–1201. www.plantphysiol.org. Copyright American Society of Plant Biologists.

by the case of P efficiency in beans, genotypes with less costly root tissue can maintain a larger total root biomass capable of acquiring more soil resources. This is important since even vigorous crops such as maize are unable to grow enough roots to explore the soil volume fully, especially during vegetative growth (Liedgens and Richner, 2001). In many drought environments, the topsoil dries before the subsoil, and, as drought progresses, roots must exploit increasingly deeper soil strata to acquire water. Genotypes capable of supporting greater root biomass would be better able to develop the extensive, deep root systems required to utilize soil water resources fully (Sponchiado *et al.*, 1989; White and Castillo, 1989). In maize, however, simple selection for root system size without regard for root costs actually decreases drought tolerance, by diverting assimilates from grain (Bruce *et al.*, 2002). Excessive root growth early in the growing season may also be counterproductive by exhausting soil water reserves before the plant is able to complete its life cycle (Richards and Passioura, 1989). Traits that affect the metabolic efficiency of root growth and soil exploration should therefore be important components of drought tolerance.

The importance of ‘cortical burden’

The metabolic cost of constructing root tissue is related to its biochemical composition, and the metabolic cost of sustaining root tissue is a function of the amount of protoplasm in that tissue and its metabolic activity, as affected by protein turnover, ion uptake, temperature, etc. (Amthor, 2000). Root anatomy is an important determinant of both tissue composition and the proportion of root tissue that is metabolically

active. In the majority of plant species, the greatest proportion of protoplasm in primary root tissue is typically found in parenchyma cells of the root cortex (Fig. 2a). Other metabolically active cells in the epidermis, endodermis, and living cells of the xylem and phloem are generally a small proportion of root volume, except in highly aerenchymatous plants such as rice, in which proportionately more protoplasm is in the stele pith, epidermis, and endodermis (Fig. 2b). Mature xylem and sclerenchyma are dead and therefore do not incur maintenance costs. Variation in the proportion of root tissue represented by cortical parenchyma, the metabolic costs of which we term 'cortical burden' (Jaramillo *et al.*, 2013), can therefore influence root costs. We have proposed that a simple measure of this is 'living cortical area' (LCA), measured in images of root cross-sections as total cortical area minus the area of root cortical aerenchyma (RCA), intercellular air space, and cell wall area (Jaramillo *et al.*, 2013). A recent study supports a role for cortical burden in root costs and water acquisition under drought stress (Jaramillo *et al.*, 2013). In this study of contrasting maize lines grown under well-watered or water stress conditions in soil mesocosms, LCA was correlated with root segment respiration, axial root growth, and plant growth under drought stress (Fig. 3). Several root anatomical phenes comprise LCA, including the number of cell files across the cortex (cortical cell file number or CCFN), the size of cortical cells (cortical cell size or CCS), and RCA.

Root cortical aerenchyma

There is increasing evidence that RCA can improve the acquisition of water and nutrients by reducing the metabolic costs of soil exploration. Although the majority of research on RCA relates to its role under hypoxia (Jackson and Armstrong, 1999; Drew *et al.*, 2000), RCA is induced by a range of abiotic stresses under normoxic conditions, including low N, P, and sulphur (S), high temperature, and drought (Drew *et al.*, 1989; Przywara and Stepniowski, 2000; Bouranis *et al.*, 2003; Evans, 2003; Zhu *et al.*, 2010a). The induction of RCA by nutrient stress led to the hypothesis that it was adaptive in infertile soils by reducing the metabolic cost of soil exploration (Lynch and Brown, 2008). This proposal was supported by a study in which RCA formation resulting from P stress, ethylene treatment, and natural genotypic variation in maize

and bean was associated with substantial reductions in the respiration of root segments and whole-root systems, as well as substantial reductions in root P content (Fan *et al.*, 2003), and greater root growth in low-P soil (Lynch, 2007). A study with the functional-structural plant model *SimRoot* (Lynch *et al.*, 1997) indicated that RCA could substantially improve the vegetative growth of plants under P stress (Postma and Lynch, 2010). After 40 d of simulated growth under P stress, RCA improved the growth of common bean (*P. vulgaris*) plants by 14% and improved the growth of maize (*Zea mays*) plants by 70%, by reducing the P content and respiration of root tissue. In a subsequent *SimRoot* study, RCA increased the growth of 40-day-old maize plants up to 55% under N stress, up to 54% under P stress, and up to 72% under K stress. The inclusion of N in the second study is noteworthy in the present context since nitrate leaching results in the accumulation of N in deep soil strata over time. In this study, RCA increased rooting depth and therefore the capture of N as a leaching resource (indeed, the benefit of RCA was greater when soil texture or precipitation accelerated nitrate leaching). This is relevant to water capture as in many environments water is concentrated in deep soil strata as drought develops. The prediction by *SimRoot* that RCA can improve rooting depth and N capture under N limitation has recently been supported in studies of maize lines with contrasting RCA phenotypes grown under low N conditions in soil mesocosms and field environments (Saengwilai, 2013). In these studies, differential RCA expression among maize recombinant inbred lines was associated with deeper rooting, better plant growth, and 60% better yield under N stress. The utility of RCA for improved water acquisition under drought was demonstrated by a study which compared maize recombinant inbred lines contrasting for RCA formation under water stress in soil mesocosms and a field rainout shelter (Fig. 4; Zhu *et al.*, 2010a). In this study, RCA formation was associated with reduced root segment respiration, deeper rooting, especially under water stress, and

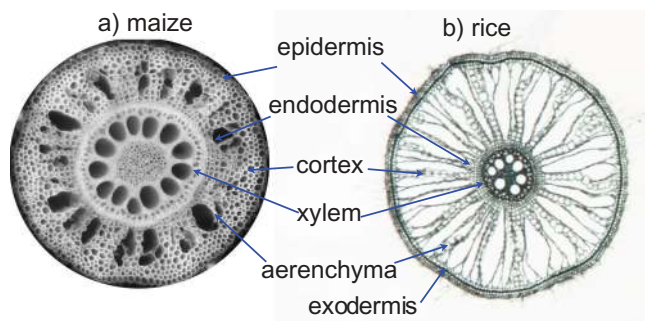


Fig. 2. Cross-section of maize (a) and rice (b) nodal roots showing anatomical features of each. Note the paucity of cortical cells in rice compared with maize, due to extensive aerenchyma development.

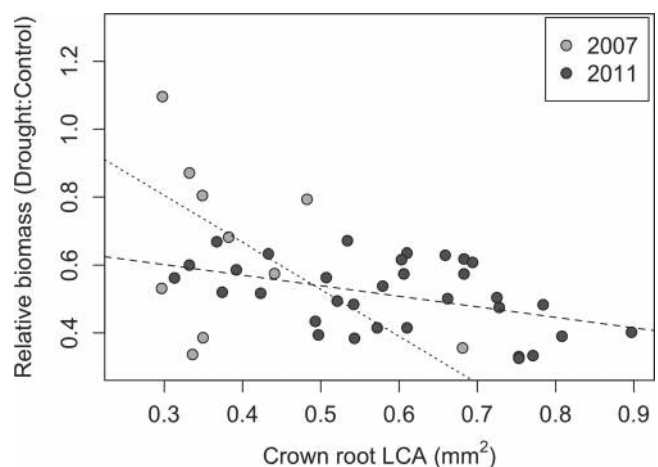


Fig. 3. Relative shoot biomass (ratio of drought to control) as a function of living cortical area of crown roots of maize grown in the greenhouse. Four recombinant inbred lines (RILs) were grown in 2007 ($P = 0.058$, $R^2 = 0.34$) and nine RILs in 2011 ($P = 0.008$, $R^2 = 0.20$). From Jaramillo RE, Nord EA, Chimungu JG, Brown KM, Lynch Jp. 2013. Root cortical burden influences drought tolerance in maize. *Annals of Botany* 112, 1–9.

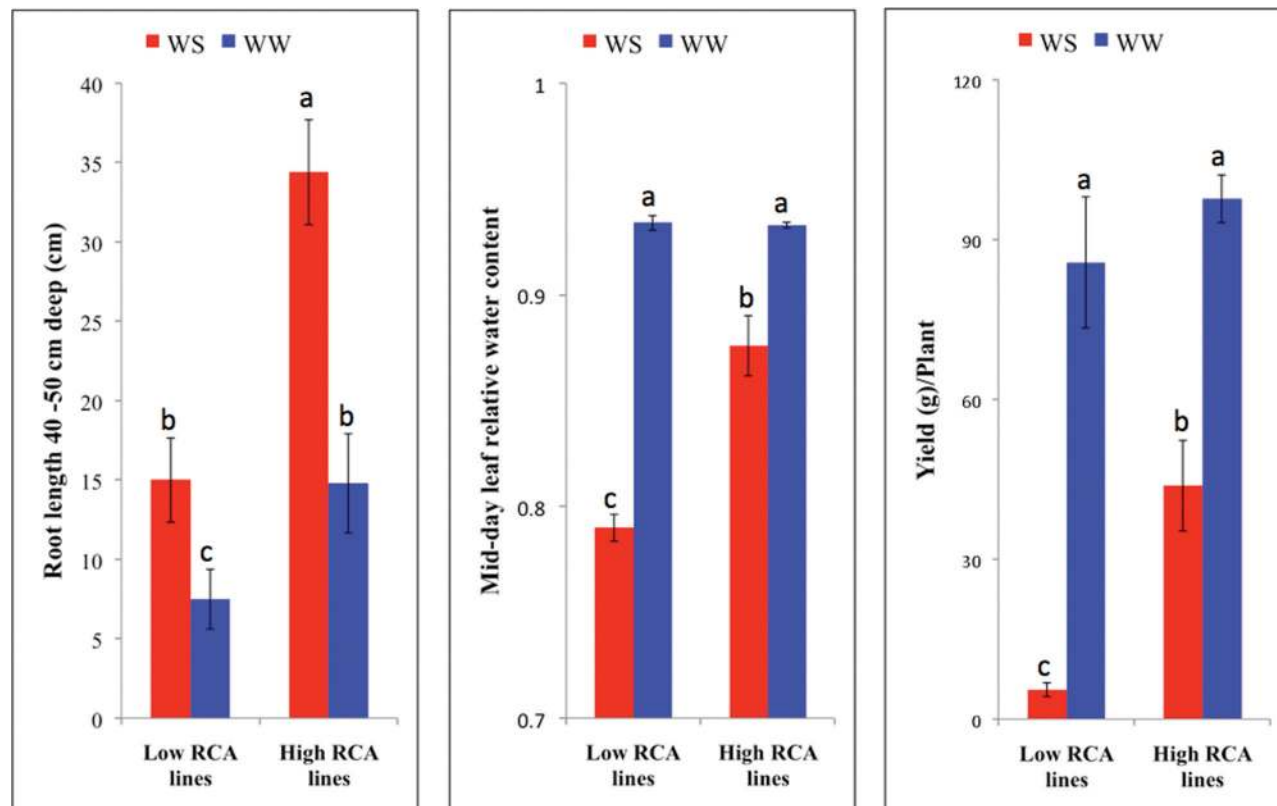


Fig. 4. Root length at a depth of 40–50 cm, mid-day leaf relative water content, and seed yield of maize plants with high or low root cortical aerenchyma (RCA) formation under well-watered (WW) or water-stressed (WS) conditions in the field. Bars having different letters within a panel are different at $P \leq 0.05$. From Zhu JM, Brown KM, Lynch Jp. 2010. Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant, Cell and Environment* **33**, 740–749. (This figure is available in colour at JXB online.)

better leaf water status, plant growth, and yield under water stress. On average, high RCA genotypes had 8-fold greater yield than low RCA genotypes under water stress.

Taken together, these studies provide strong evidence that RCA improves soil resource acquisition by reducing the metabolic cost of soil exploration. The combination of results from *SimRoot*, greenhouse mesocosms, and field environments in South Africa and the USA is less vulnerable to misinterpretation than would be results from any one of these environments in isolation. Field studies can be influenced by complex environmental interactions of root phenes with soil properties (including soil biota), which are simplified or absent in greenhouse mesocosms. The agreement of results from *SimRoot* modelling and empirical studies is noteworthy as *SimRoot* is a greatly simplified ‘environment’ focusing primarily on soil resource acquisition as influenced by plant resource allocation and root structure. The fact that *SimRoot* correctly predicted the value of RCA for soil resource acquisition by considering root metabolic costs is a rigorous demonstration that the quantitative logic of this hypothesis is valid, considering root growth dynamics and fluctuating soil resource availability in time and space. Although RCA formation was beneficial in unstressed plants in the *SimRoot* studies, both *SimRoot* and empirical studies show that the benefit of RCA increased with greater water or nutrient stress. This is because a relatively small increase in the acquisition of a limiting resource can have relatively large effects on plant

growth, because of the autocatalytic interaction of greater soil resource capture with greater photosynthesis and therefore greater root growth (Wissuwa, 2003).

A recent study reported substantial genetic variation for RCA formation and other root traits among 256 *Zea* accessions spanning a range of wild species and landraces from diverse provenances, emphasizing stressful soil environments (Burton *et al.*, 2013). Quantitative trait loci (QTLs) have been identified for RCA abundance in maize using a *Z. mays* × *Z. nicaraguensis* backcross population (Mano and Omori, 2008, 2009), and more recently in several *Z. mays* recombinant inbred populations (Burton, 2010), and in association studies (Saengwilai, 2013) using a diversity panel developed for use in temperate climates (Hansey *et al.*, 2011).

Considering the substantial benefits of RCA for soil resource capture, the large genetic variation for RCA formation in maize suggests that RCA formation entails trade-offs for plant fitness. Most obviously, the conversion of living cortical tissue to air space will result in the loss of normal cortical functions, including radial transport of water and nutrients to the stele, buffering of toxic ions (notably Na and Cl under salinity stress), and mycorrhizal habitat. The loss of cortical cells may also affect the biophysical properties of the root, which could be important in the penetration of hard, dry soil. Aerenchyma lacunae may create low resistance pathways for the spread of root pathogens once they enter the cortex. There is very little

information about potential RCA trade-offs in the literature. One study investigating the relationship between RCA and resistance to radial compression, such as could occur with trampling in pastures, showed that RCA did not reduce mechanical strength in the presence of multi-seriate layers in the outer cortex, a characteristic found in the graminaceous and cyperaceous species tested (Striker *et al.*, 2007). RCA formation reduces radial hydraulic conductance (Fan *et al.*, 2007) and can reduce the radial movement of nutrients in maize roots (Hu *et al.*, 2014). In this regard it is important to consider the spatial distribution of RCA formation (Fig. 5). RCA forms in mature root tissue behind the zone of active root elongation and root hair formation. As such the region of RCA formation is behind the region where most of the acquisition of immobile resources such as P, K, and ammonium occurs. Nitrate and water are more mobile soil resources, and RCA formation may interfere with the mass flow of these resources to mature roots, although, under conditions of low water or nitrate availability, the capture of these resources may also be localized largely to the root tips. Therefore, even though RCA formation reduces the radial transport of water and nutrients in mature root tissue, the effects this may have on soil resource acquisition by the whole root system are unclear and merit investigation. Information about other potential trade-offs for RCA formation, including biotic interactions, is lacking.

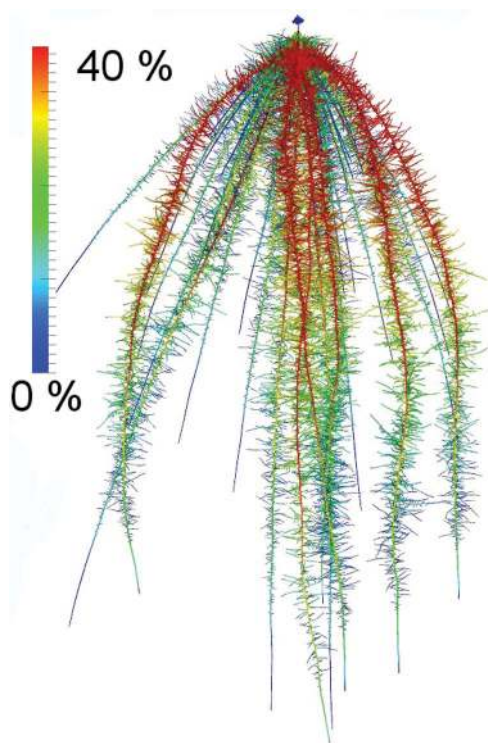


Fig. 5. Spatial distribution of root cortical aerenchyma in simulated maize plant 40 d after planting, with hotter colours representing more aerenchyma. From Postma JA, Lynch JP. 2011. Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiology* 156, 1190–1201. www.plantphysiol.org. Copyright American Society of Plant Biologists.

Root cortical senescence

Root cortical senescence (RCS; sometimes called root cortical death) is a phenomenon widely reported in cereals, particularly wheat and barley. This phenomenon has been characterized using various staining techniques and by DNA fragmentation (Lascaris and Deacon, 1991a; Wenzel and McCully, 1991; Liljeroth and Bryngelsson, 2001). Careful examination of the vitality of cortical cells in wheat and barley showed that RCS resulted in sloughing of most cortical cells in older roots, but that the root cortex of younger roots did not senesce (Wenzel and McCully, 1991), contradicting numerous early reports of cortical senescence of very young plants, assessed by acridine orange staining (Henry and Deacon, 1981; Kirk and Deacon, 1987). In later work, a pressure probe was used to test cortical cell turgor, revealing a progressive decline in turgor with distance from the root apex and the association of significant turgor loss with loss of visible nuclei and reduced root diameter (Bingham, 2007).

RCS has several similarities to formation of lysigenous RCA. Both phenomena involve lysis of cortical cells preceded by molecular changes characteristic of programmed cell death, such as DNA fragmentation and autophagy (Lingua *et al.*, 1999; Gunawardena *et al.*, 2001; Liljeroth and Bryngelsson, 2001; Jiang *et al.*, 2010). Both processes are accelerated when mineral nutrients are insufficient (Gillespie and Deacon, 1988; Drew *et al.*, 1989). However, RCS and RCA formation have distinct characteristics; for example, nuclei persist in the innermost cortical layer during RCS, while these are the first to be lost during RCA formation (Henry and Deacon, 1981; Deacon *et al.*, 1986), and silver ions, which inhibit ethylene action, reduce RCA but not RCS formation (Drew *et al.*, 1981; Lascaris and Deacon, 1991a). In addition to loss of the cortex, RCS involves sloughing of the outer layers of the root, removing the barriers of the epidermis and exodermis; these remain intact during RCA formation. RCA formation in maize is not followed by RCS (Wenzel and McCully, 1991) and it appears that species differ in their propensity to one or the other.

Presumably RCS formation would produce metabolic savings similar to those discussed for RCA, since living cortical cells are lost, allowing their contents to be redistributed and eliminating their respiratory maintenance costs. It has been proposed that formation of RCS, like RCA, would reduce the P cost of the roots, and therefore benefit the plants by permitting recycling of P from the senesced cortical cells (Robinson, 1990), but this hypothesis has not been tested. Unlike RCA, RCS would increase the radial conductivity of water and nutrients, since the layers outside the endodermis have now been lost. Of these, the epidermis/exodermis is most limiting for radial conductivity, but the cortex imposes some additional limitation (North and Nobel, 1995, 1996). The RCS progression rate has been associated with increased susceptibility to diseases, though diseases have not been shown to increase the rate of RCS (Yeates and Parker, 1986; Kirk and Deacon, 1987; Lascaris and Deacon, 1991b). On the other hand, colonization of leek roots with the mycorrhizal fungus *Glomus mosseae* slowed the progression of RCS (Lingua *et al.*, 1999).

Genetic variation has been observed for RCS formation in wheat and barley (Henry and Deacon, 1981; Deacon and

Lewis, 1982; Liljeroth, 1995), indicating that it could serve as a breeding target if the utility of this trait is demonstrated. However, much more research is needed before this trait could be deployed. It may be possible to phenotype large populations using RootScan (Burton *et al.*, 2012b) or similar programs, but this has not been attempted. The value of the trait, its trade-offs, and the optimal expression of RCS in various environments are unknown. In terms of genetics, nothing is known of the genes controlling the process and there have been no genetic maps generated or identification of genes likely to be responsible.

Cortical cell file number

By analogy with RCA, variation in CCFN should also be expected to reduce the metabolic costs of soil exploration, and thereby influence water acquisition from drying soil. CCFN ranged from 6 to 16 in a wide survey of the genus *Zea* (Burton *et al.*, 2013). Such variation should be closely related to root segment respiration, and therefore rooting depth, and water acquisition, as observed for variation in RCA. A potential trade-off of this phene for water acquisition is that it would reduce root diameter, which is related to the ability to penetrate hardening, drying soil (Clark *et al.*, 2003, 2008).

Synergism with architectural traits

The utility of RCA and probably other root anatomical phenes for soil resource acquisition will be affected by the external environment as well as the plant phenotype in which it is expressed. In the *SimRoot* studies of RCA (Postma and Lynch, 2010, 2011), both types of interactions were observed. RCA was generally more useful for N capture in environments with greater leaching, caused by changes in soil texture or precipitation, since the benefit of RCA for the rapid development of deep roots had greater effects on N capture when N leaching was more rapid. This is probably similar for water capture under drought. RCA may be more advantageous for water capture in drought environments in which water is more available at depth because of the rate of surface soil dehydration or soil textural stratification, compared with soils in which water availability is less stratified by depth. RCA was more beneficial for soil resource capture in maize phenotypes with abundant root branching (Postma and Lynch, 2011) or a greater number of nodal roots (York *et al.*, 2013), since in such phenotypes root metabolic costs are relatively more important than in phenotypes with less branching. Such phene interactions are probably common, and important for plant fitness, although they are poorly understood (York *et al.*, 2013). The number of potential phene interactions and phene×phene×environment interactions of interest in this context is quite large, which calls for *in silico* optimization approaches (York *et al.*, 2013).

Traits that influence water transport

Xylem vessel diameter

Xylem vessel traits are important because of their direct effect on axial water conductance. A breeding programme

conducted in Australia for dryland spring wheat production targeted seminal root xylem vessel diameter as a method for improving water use efficiency (Passioura, 1983; Richards and Passioura, 1989). In the targeted agroecosystem, the spring wheat crop relies on stored water, and there is a risk of stored water depletion before grain fill is complete. The concept was that reduced xylem vessel diameter in the seminal (seedling) roots would reduce water use early in the season, conserving available water to support grain set and yield (Richards and Passioura, 1981a, b). Since water transport in xylem vessels is proportional to the fourth power of the vessel radius (Hagen–Poiseuille law), reducing the diameter of the largest xylem vessels would have a strong effect on water flow through the root system by reducing axial conductance. Under ample water availability, the development of the nodal root system would greatly increase axial conductance and support the evapotranspiration needs of larger shoots (Richards and Passioura, 1981a; Passioura, 1983). Genetic variation for xylem vessel diameter and number was demonstrated in wheat, and heritability of xylem vessel diameter was high (72%) (Richards and Passioura, 1981a, b). Selection for smaller xylem vessel diameter resulted in a small improvement of wheat yield under drought without a yield penalty in wetter seasons (Richards and Passioura, 1989; Watt *et al.*, 2013). This approach was later superseded by alternate methods of selecting for effective use of water (Blum, 2009).

In most agroecosystems, where water is available at depth throughout the season, improving water acquisition may require increases in axial conductivity. Larger xylem vessel diameter has been used as a selection criterion for breeding rice in Asia, where the goal is improved water acquisition and transport rather than conservation. It was proposed that larger xylem vessels could improve water uptake and yield, especially if combined with deep roots (Yambao *et al.*, 1992; Kondo *et al.*, 2000). Root diameter, which has repeatedly been positively associated with drought avoidance in rice (Gowda *et al.*, 2011), is positively correlated with the size of late metaxylem in rice (Yambao *et al.*, 1992). As in wheat, xylem vessel diameter was shown to be highly heritable and genetically variable, and QTLs regulating the size and number of late metaxylem have been identified using a biparental population derived from *indica* and *tropical japonica* parents (Uga *et al.*, 2008). A study of *aus* and hill cultivars showed a positive relationship between xylem vessel diameter and drought resistance (Haque *et al.*, 1989), while another study using five rice cultivars failed to show a relationship between xylem vessel diameter and response to drought (Kondo *et al.*, 2000). However, given the number of other important factors for drought response, the possible contribution of xylem vessel diameter to improving water acquisition and utilization, and its interaction with other traits, such as deep roots, deserves additional research.

Xylem vessel number and diameter are affected by the environment as well as by genetics. Late metaxylem vessel diameter in rice declines with the imposition of drought, a change that was suggested to reduce the risk of cavitation (Henry *et al.*, 2012). Likewise drought causes reduction in late metaxylem vessel diameter in the grass *Paspalum dilatatum* (Vasellati *et al.*, 2001) and grapevines (Mapfumo *et al.*, 1994).

Deep roots of trees had larger late metaxylem vessel diameter than shallow roots (McElrone *et al.*, 1999).

Given the fact that radial water conductance is considered to be more limiting for water movement than axial conductance, is metaxylem vessel diameter a reasonable target for improving water acquisition; that is, drought avoidance? For water conservation in cereals, early work showed that drought can reduce nodal root development, and, since monocots lack secondary growth, axial water transport depends on the few late metaxylem vessels of the seminal roots (Passioura, 1983). Restricting their size would then restrict overall hydraulic conductance and therefore water usage. Whether increasing the size of late metaxylem vessels in other plants would improve water uptake depends on other root traits and the agroecosystem in question.

Cell wall modifications: suberization and lignification

Suberization and lignification of root cell layers can restrict the movement of water, nutrients, microorganisms, and even gases into and within the root. The endodermal and exodermal layers, which are, respectively, the innermost and outermost layers of the cortex, contain Casparian bands that are suberized and lignified, and may additionally develop suberin lamellae (Ma and Peterson, 2003). These cell wall modifications regulate the movement of water and ions, mainly by reducing apoplastic transport (Steudle, 2000; Enstone *et al.*, 2003; Ranathunge *et al.*, 2005). Many wetland plants, including rice, develop a barrier to radial oxygen loss through suberization of the hypodermis (Colmer, 2003; Garthwaite *et al.*, 2008).

Suberization and lignification of the endodermis have often been observed to increase in stressed roots (Hose *et al.*, 2001); for example, in drought-stressed rice, suberization of the endodermis increased and that of the sclerenchyma layer decreased (Henry *et al.*, 2012). Rice cultivars varied in the amount of endodermal suberization under drought, with more drought-tolerant lines having a stronger response (Henry *et al.*, 2012). Water retention under drought may be improved by increased suberization of the endodermis (Henry *et al.*, 2012), while Na⁺ uptake was negatively associated with endodermal and exodermal suberization in rice (Krishnamurthy *et al.*, 2011). The endodermis was an important regulator of hydraulic conductance in a comparison of several soybean varieties, though this relationship was based on dimensions and not directly on suberization (Rincon *et al.*, 2003). An examination of five *Arabidopsis* accessions with different root hydraulic conductivities did not show any relationship between suberization patterns and hydraulic profiles (Sutka *et al.*, 2011). Another study using *Arabidopsis* mutants with altered suberin composition showed that reduced tissue content of certain polymers was associated with increased radial conductivity (Ranathunge and Schreiber, 2011), suggesting a potential for genetic variation in this trait. Clearly more research is needed on the role of suberization in controlling hydraulic conductivity and the movement of ions.

In order for suberization and lignification to become selection targets in plant breeding, much more needs to

be learned about the environmental and genetic control of development of apoplastic barriers in various root tissues. For example, what are the advantages and disadvantages of earlier and/or more intense suberization of root endodermal, exodermal, and sclerenchyma layers under stressed and unstressed conditions? Would plasticity of these traits be advantageous or not? How do they interact with other, non-anatomical traits affecting radial conductivity, such as aquaporin expression? What types of genetic factors control these processes and how could they be manipulated in a breeding programme?

Rhizosheaths

Rhizosheaths are composed of soil particles bound to the root by root hairs, microorganisms, and mucilages to form a distinct soil domain around the roots of grasses and certain other plants (McCully, 1999). They form on young, active regions of the root where the epidermis is still intact, though not at the growing root tip. They are lost from the more mature portions of the root, and their presence is indicative of immature, non-conducting late metaxylem vessels (McCully, 1995). Rhizosheaths are thought to improve the hydration of roots in dry sandy soils (North and Nobel, 1997), but mesophytic plants as well as xeric plants form rhizosheaths (Watt *et al.*, 1994; McCully, 1999; Haling *et al.*, 2010a). The mucilage in the rhizosheath help this domain to hold more water than the bulk soil (Young, 1995), and, even under drought, the discrete structure of the rhizosheath prevents an air gap from forming when the soil shrinks from dehydration (McCully, 1995; Walker *et al.*, 2003). These features may assist in nutrient uptake; however, this possibility has received little attention, apart from work by Nambiar (1976) suggesting that the rhizosheath improved zinc uptake in several grasses. In addition, the fact that the underlying root tissue lacks significant hydraulic conductivity casts doubt on the role of the rhizosheath for plant water relations (Guinel and McCully, 1986), although hydraulic isolation of the root tips may have survival value in dry soil. Another suggested function for the rhizosheath is management of microbial populations via the activity of border cells, which remain alive in this region (Hawes *et al.*, 1998).

The main plant features that have been demonstrated to affect formation of the rhizosheath are root hairs and mucilage production. Mucilage is produced by border cells and persists in the rhizosphere as the growing tip moves beyond the zone of production; it is this mucilage that binds the rhizosheath together (Vermeer and McCully, 1982; McCully, 1995). Very little is known about the genetics of mucilage production, and mucilages from the root cap and border cells are distinct from those of the epidermis (Vermeer and McCully, 1982), making investigation of this phene more complex.

Root hairs are particularly important in determining the size of rhizosheaths, and root hair length was directly related to rhizosheath mass in wheat and barley (Haling *et al.*, 2010b; Brown *et al.*, 2012b). Substantial research supports an important role for root hairs in P acquisition (Gahoonia and

Nielsen, 1997, 1998, 2004; Bates and Lynch, 2001; Zhu *et al.*, 2010b; Brown *et al.*, 2012a, b), and additional work shows their importance under combined stresses of low P plus drought (Brown *et al.*, 2012b) and low P plus soil compaction (Haling *et al.*, 2013). Genetic variation in rhizosheath formation on acid soil in wheat and barley (Haling *et al.*, 2010b) was associated with the aluminium tolerance of the root hairs (Delhaize *et al.*, 2012), demonstrating the importance of root hairs for rhizosheath formation. Selecting for longer root hairs with greater persistence would be expected to increase the size of the rhizosheath.

Another possible anatomical feature that could affect abiotic stress tolerance via rhizosheath processes is the position of xylem vessel maturation along the root axis. Since rhizosheaths develop only over zones of immature xylem vessels and are lost after the xylem matures (McCully, 1999), altering the length of the immature xylem vessel zone should also alter the length of the rhizosheath. We are not aware of any research showing genetic variation for this trait.

Traits that improve penetration of hard soil

Mechanical impedance to root growth is one of the most important factors determining root elongation and proliferation within a soil profile. Genetic variation within and between species has been reported for the capacity of roots to penetrate hard soil (Materchera *et al.*, 1991; Bengough *et al.*, 2006) and access water in deep soil layers. For example, rice cultivars that were able to penetrate hardpans had better access to water and better drought avoidance (Clark *et al.*, 2002), indicating that the mechanical impedance experienced by roots is a major limitation to root growth in drying soil. There is a strong relationship between root diameter and the ability of the root to penetrate hard soil (Materchera *et al.*, 1991). Thicker roots are more resistant to buckling and deflection when encountering hard soil (Whiteley *et al.*, 1982; Clark *et al.*, 2008), explaining why thicker roots penetrate better in strong soils. In addition, thick roots may relieve the axial stress to the growing root tip when the root encounters hard soil (Abdalla *et al.*, 1969; Hettiaratchi, 1990). The reduction of axial stress results from soil loosening at the root tip due to radial expansion of a cavity. The relief of axial stress by thick roots depends on the level of soil–root friction, which is influenced by the presence of a slippery coating of detached border cells and mucilage (Kirby and Bengough, 2002).

In addition to root diameter, root hairs also play a role in root penetration of hard soils. Haling *et al.* (2013) proposed that root hairs improve root penetration in mechanically impeded soil. Studies involving barley lines with and without root hairs show that lines with root hairs were able to penetrate the mechanically impeded soil. Root hairs appear to help roots to penetrate into mechanically impeded soil layers by providing anchorage to the growing root tip, but do not necessarily increase the root elongation rate within the layer. One potentially relevant trait, hardly ever considered in relation to root penetration, is the genotypic variation in ability of roots to locate cracks and channels in the soil, a characteristic potentially very relevant to the penetration of structured subsoil (McKenzie *et al.*, 2009).

Root architecture phenes may also play a role in root penetration of hard soils. Steep root angles are associated with deep rooting and offer advantages in drying soils by enhancing water acquisition in deep soil layers, while shallow root angles are associated with improved acquisition of less mobile soil resources such as P (Lynch, 2013). The angle of incidence of a root at a strong layer influences root penetration (Dexter and Hewitt, 1978). Thus, steep roots have a high likelihood of penetrating horizontal strong soil layers (Whalley *et al.*, 2012). As noted above, synergisms between root growth angle and anatomical phenes affecting the penetration of hard soil should be expected, but are poorly understood.

Optimal versus maximal water acquisition from drying soil

Maintenance of soil exploration is an imperative for annual plants in drying soils. In many environments, drying soil marks the beginning of an extended dry season, in which an annual plant must maintain soil exploration to capture water that is receding to deeper soil strata. Several of the anatomical phenes discussed above directly sustain soil exploration, such as phenes that reduce root metabolic cost, and phenes that improve penetration of hard soil. In the context of the maintenance of soil exploration by individual root axes, optimal water acquisition is likely to be significantly less than maximal water acquisition. Most directly this is because of the need for the growing root axes to maintain internal water availability sufficient for turgor and elongation into hardening soil. A benefit related to reduced water acquisition is that moisture remaining in the soil surrounding the root will facilitate soil penetration by the root axes and lateral roots. The hydraulic isolation of rhizosheaths, delayed xylem maturation, and increased suberization of the endodermis may be viewed as mechanisms to reduce the desiccation of growing root tips and their surrounding rhizosphere, which is needed for sustained soil exploration and water acquisition. Reduced water supply to shoots under drought also has additional benefits under terminal drought, in the short term reducing leaf water status and stomatal conductance during periods of peak evaporative demand, thereby improving the integrated daily water use efficiency of photosynthesis, and in longer time scales by reducing shoot growth and therefore the demand for water as well as shoot/root biomass partitioning. Anatomical phenes that reduce the hydraulic conductance of root systems under drought may therefore be important both for sustaining soil exploration and water capture and for improving the economy of water use by shoots by improving water use efficiency and reducing overall water demand.

Breeding prospects/research needs

Almost all of the traits discussed above show genetic variation among crop genotypes, and thus could be deployed in crop breeding programmes if their utility can be established for drought tolerance. The utility of several traits is fairly well established and they can be deployed at least in pilot efforts.

The slowing of crop water use by selecting for reduced xylem vessel diameter is an early example of the deployment of root traits in crop breeding (Richards and Passioura, 1989). Root hair length (and root hair density, which is usually correlated with root hair length) has clear value for the acquisition of P and probably other diffusion-limited nutrients such as K and ammonium, and may improve root penetration of hard, drying soil. RCA appears to be a useful adaptation to suboptimal availability of both nutrients and water in maize. The utility of the other traits discussed above is more speculative and would require validation before deployment in breeding programmes.

An obstacle to the deployment of root phenes in crop breeding, especially anatomical root phenes, is the difficulty in evaluating root phenotypes of a large number of individual plants. Field excavation of root systems can be conducted on a fairly large scale (Trachsel *et al.*, 2011), but this must be followed by anatomical analysis, which, despite the availability of image analysis tools, remains tedious. Several anatomical phenes may be amenable to phenotypic evaluation in young plants grown under controlled conditions (Burton *et al.*, 2012a, b). Identification of genetic markers for anatomical phenes would greatly facilitate their use in breeding programmes (Uga *et al.*, 2008; Lynch, 2011; Henry *et al.*, 2012; Henry, 2013; York *et al.*, 2013).

A more challenging obstacle is the need to understand the utility of specific root phenes in the context of specific agroecosystems and specific phenotypic backgrounds, namely the fitness landscape (Lynch and Brown, 2012). As noted above, there are important fitness trade-offs for many root phenes, and important environmental and phenotypic interactions that affect the utility of any given root phene for drought tolerance. These are very poorly understood at present. The utility of anatomical phenes for water acquisition will depend on the spatiotemporal dynamics of soil water availability. Because of the complexity of the soil environment and the large number of potential interactions and scenarios, *in silico* approaches, for example via functional–structural plant models such as *SimRoot*, will be important tools in concert with empirical studies. This challenge will require expertise in plant biology, rhizosphere microbiology, and soil science that span traditional funding and training foci. Most importantly, this challenge calls for renewed emphasis on understanding the plant phenome.

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