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REGULAR ARTICLE

Morphological and architectural development of root systems in sorghum and maize

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Abstract Root systems determine the capacity of a plant to access soil water and their architecture can influence adaptation to water-limited conditions. It may be possible to associate that architecture with root attributes of young plants as a basis for rapid phenotypic screening. This requires improved understanding of root system development. This study aimed to characterise the morphological and architectural development of sorghum and maize root systems by (i) clarifying the initiation and origin of roots at germination, and (ii) monitoring and quantifying the development of root systems in young plants. Three experiments were conducted with two maize and four sorghum hybrids. Sorghum produced a sole seminal (primary) root and coleoptile nodal roots emerged at the 4th–5th leaf stage, whereas maize produced 3–7 seminal (primary and scutellum) roots and coleoptile nodal roots emerged at the 2nd leaf stage. Genotypic variation in the flush angle and mean diameter of nodal roots was observed and could be considered a suitable target for large scale screening for root architecture in breeding populations. Because of the relatively late appearance of nodal roots in sorghum, such screening would require a small chamber system to grow plants until at least 6 leaves had fully expanded.

Keywords Nodal root · Root angle · Root architecture · Scutellum · Seminal root

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Introduction

The capacity of plant roots to access available soil water is critical to crop adaptation in water-limited environments (Ludlow and Muchow 1990). This is especially important in species such as sorghum (*Sorghum bicolor* L. Moench.) and maize (*Zea mays* L.) that are frequently grown in such environments. Recent studies on maize have suggested that change in root system architecture and associated water capture likely underpins historical yield advance from breeding in the US Corn Belt (Hammer et al. 2009). The importance of root system attributes in sorghum has also been implicated in modelling studies that quantified the relative adaptive advantage of sorghum over maize in

water-limited conditions (Sinclair and Muchow 2001). Extensive genetic variation has been observed in sorghum root systems (Jordan et al. 1979) and studies on other species have highlighted the critical role of root system architecture in crop adaptation. In wheat (*Triticum aestivum* L.), Manschadi et al. (2006) showed that two genotypes with contrasting tolerance to water limitation in field experiments differed in root system architecture. The drought tolerant wheat had a more compact and uniform root system with greater root length at depth that supported enhanced water extraction under terminal drought conditions.

The temporal development of root system architecture is determined by the nature of the root system and its rate of progression into the soil. In cereals, such as sorghum and maize, the root system is formed from the seminal roots that appear at germination and the nodal, crown or adventitious roots that arise later from nodes of the shoot (Esau 1967). Seminal roots play an important part in initial water and nutrient uptake and establishment of seedlings, whereas nodal roots dominate during the later stages of growth. Studies in sorghum and maize throughout their life cycles indicate that the root system grows into the soil at about 2–3 cm day^{-1} (Dardanelli et al. 1997; Manschadi et al. 2008; Robertson et al. 1993; Whish et al. 2005). Root system development of maize has been relatively well studied compared to that of sorghum, but there has been inconsistency in the nomenclature used for root types in maize. Roots have been variously described as primary, seminal, nodal, or axile and have often been associated with descriptors of their point of origin, such as radicle, coleorhiza, scutellar node, coleoptilar node, and mesocotyl (Cahn et al. 1989; Ho et al. 2003; Hochholdinger et al. 2004; Hund et al. 2007; Mollier and Pellerin 1999; Tillich 1977).

It may be possible to associate mature plant root system architecture with the nature of root system development in young plants. This would provide an opportunity for rapid and effective phenotypic screening of breeding populations for desirable root system properties. A number of studies across species (Kato et al. 2006; Lynch and van Beem 1993; Manschadi et al. 2008; Nakamoto and Oyanagi 1994) have indicated that the angle at which root axes appeared at the seedling stage was associated with subsequent root system architecture and acquisition of soil resources. Early screens for root architecture have been developed for maize (Hochholdinger et al. 2004; Hund et al. 2009), barley (*Hordeum vulgare* L.) (Hargreaves et al. 2009), and wheat (Bengough et al. 2004; Manschadi et al. 2008). An improved understanding of the development and growth of root systems in young plants of sorghum is required in order to develop specifications for a rapid phenotyping system for root architecture. Comparison of development of root systems in sorghum and maize would inform this process for sorghum and clarify inconsistencies for maize.

Hence, the aim of this study was to characterise the morphological and architectural development of sorghum and maize root systems by (i) clarifying the initiation and origin of roots at germination, and (ii) monitoring and quantifying the development of root systems in young plants.

Material and methods

Plant material and experimental overview

The experiments included four sorghum and two maize hybrids. The sorghum hybrids resulted from a factorial combination of two female (ATx642, previously known as B35, and AQL39) and two male (RQL12 and RQL36) parents. ATx642 and RQL12 are known to be sources of the stay-green trait while RQL36 and AQL39 are senescent (R.G. Henzell Pers Comm). The resulting F1 hybrids (ATx642/RQL12, ATx642/RQL36, AQL39/RQL12, and AQL39/RQL36) differ in stay-green and in the capacity to yield under terminal water deficit (Borrell et al. 2000). The two maize hybrids, 3394 and 3343, have some ancestry in common (Smith et al. 2004), but 3343 has unstable dryland yield (Anderson et al. 2004) and is considered to be more drought susceptible.

The hybrids were grown in three experiments, conducted under semi-controlled conditions at The University of Queensland, Brisbane, Australia (27° 23′S, 153°06′E). Experiment 1 (Exp1) aimed to characterise the initiation and development of roots immediately after germination. Experiment 2 (Exp2) aimed to characterise root system development during growth of young plants. Experiment 3 (Exp3) aimed to quantify root elongation rate.

Experiment 1: Initiation of roots at germination

The objective of this experiment was to characterise the nature of early root system development in

sorghum and maize and determine any qualitative differences between species. Ten (maize) or twenty (sorghum) seeds per hybrid were placed in a petri dish on filter paper moistened with distilled water and left to germinate at 28°C in an incubator, in the dark. As root systems had outgrown the petri dishes 2 days after germination, one seed per hybrid was wrapped in thick paper towel and moistened with distilled water daily to allow observation of roots from 3 to 7 days after germination. Germinated seeds were photographed every 24 h with an Olympus SZH10 stereo microscope at 0.7 magnification for the first 2 days, and 1.0 magnification for the next 2 days. The final photograph (7 days after germination) was taken with a Panasonic NVGS400GN mini DV digital video camera. For all photographs, seeds were positioned in a way that best exposed the initiation and development of the different root types. Observations on origin and timing of initiation of different types of roots were made daily.

Experiment 2: Root system development during early growth

The experiment was conducted in a glasshouse with plants grown in purpose built root observation chambers that were 60 cm high, 40 cm wide, and 3 cm thick. Transparent perspex (8 mm thick) sides were used to enable viewing and scanning of roots. The perspex sheets were screwed to the metal frame of the chamber to allow easy removal at harvest. Each chamber was filled with 10.7 kg air dried soil (70% black alluvial clay loam mixed with 30% sand) and watered to field capacity a few days before sowing. Each chamber was first wrapped in black plastic sheeting to prevent exposure of the roots to light, and then encompassed in aluminium-coated sheeting to reflect incoming light and minimise effects on soil temperature. Liquid fertilizer (Aquasol) was applied to each container 2 days before planting to ensure nutrients were non-limiting.

Seeds of the 6 hybrids were pre-germinated in petri dishes in an incubator at 28°C (as for Exp 1). Two pre-germinated seeds were planted in each chamber and thinned to one plant 2 days later. The experiment consisted of three replications and five harvests for each hybrid. As there were only sufficient purpose-built chambers for one replication, the experiment was conducted in three separate runs, with each run considered a replication. Planting dates of the pre-germinated seeds for each replication were 11 October 2006, 25 November 2006, and 22 February 2007. The first harvest occurred when two leaves had fully expanded and subsequent harvests were conducted each time a new leaf had fully expanded on the main culm, until the six leaf stage. These harvests were denoted L2–L6. The experiment was laid out as a split plot design, with hybrids as the main plot and the five harvest times as subplots. The five chambers per subplot were arranged in plastic boxes, giving a plant-to-plant spacing of 5 cm.

Prior to each harvest, the area of each leaf blade on every plant was estimated in situ from the measured length and width, multiplied by a scaling factor of 0.69 for sorghum (Lafarge and Hammer 2002) and 0.75 for maize (Turner 1975). At harvest, the shoot of each plant was cut at the base of the stem, and separated into stem (including leaf sheath) and leaf blade fractions. After removing the shoot, each chamber was laid flat. The top perspex plate was removed and a pinboard, with similar dimension to the chamber, was placed on top of the exposed soil. The pinboard consisted of 3 cm long nails, positioned in a 2×2 cm grid on a plywood base that was painted black. The pinboard was pushed into the soil block and the soil with root system was carefully transferred to it without disturbing the root system architecture. The soil was then washed from the pinboard using a very slow water spray to minimise disturbance of the root system. After all soil had been removed, the intact washed root system was imaged with a digital camera mounted on a tripod. The images were converted to high-contrast black and white images using Adobe Photoshop Elements 2.0 software. After imaging, the number of seminal and nodal roots was recorded for each plant. The length of both the seminal and nodal roots was measured with a scale and their diameter measured 1 cm below the seed with a verniere digital caliper. The angles of the initial root segments of the first and second flushes of nodal roots were measured from the images, relative to the vertical axis, using in-house software, specifically designed for this purpose, and averaged for both sides of the plant. Dry matter of each plant part (leaf blade, stem, roots) was obtained after drying in a dehydrator for 4 days at 60°C.

Experiment 3: Root elongation rate (RER)

The experiment was conducted in a glasshouse using 15 cm diameter PVC pipes cut to four lengths (30, 60, 90, and 120 cm). The base of each pipe was

fitted with steel mesh (2 mm) in order to observe the time the root tip appeared. Each pipe was filled with a soil mixture (70% black alluvial clay loam mixed with 30% sand) and the profile watered. After 24 h draining, 500 ml of liquid nutrient solution containing N:P:K (21:5:17) was added. The containers were lightly watered throughout the experiment when the surface soil appeared dry and before any signs of water limitation occurred. The PVC pipes were suspended in a purpose-built rack, such that their tops were all at a similar level.

Seeds of the six hybrids were soaked overnight and then pre-germinated in an incubator at 28°C in petri dishes for 2–3 days. Two germinated seeds were planted in each container on 10 June 2006 and thinned to one plant after 2 days. The experiment was laid out as a split plot design with three replications, with hybrids as the main plots and pipe length (harvest time) as the subplots.

The containers were monitored daily for the appearance of root tips at the bottom. Once root tips were observed on more than 50% of pipes of a given length, all pipes of that length were removed and plants harvested. Root elongation rate (cm d^{-1}) was calculated as the slope of the relationship between soil depth (container length) and date of root tip appearance. No distinction could be made between seminal and nodal roots.

Statistical analysis

Analyses of variance on shoot and root parameters were performed using the procedure for split-plot design in GENSTAT v9.0, after checking the homogeneity of variance. Relationships between selected parameters were examined using regression analysis and differences tested using analysis of covariance.

Results

Maize and sorghum differed in origin of seminal and nodal roots (Exp1)

Maize and sorghum plants showed consistent differences in the origin and number of seminal and nodal roots. Differences among hybrids within species, however, were small. Therefore, this section focuses on qualitative differences between species only, with photographs depicting results for representative plants of each species.

In maize, a primary root (PR), and a coleoptile (C) had emerged by 2 days after germination (Fig. 1a). The PR emerged from the lower end of the seed through the coleorhiza (CO), whereas the C emerged at the upper end of the seed. Scutellar seminal roots (SSR) had also emerged from the scutellum (S) by 2 days after germination (Fig. 1a) and four SSR (two appearing in front of the C and two behind) were evident by day 3 (Fig. 1b–c). The seminal root system in maize thus consisted of the PR and the SSR. The SSR originating from the S might be misinterpreted as nodal roots. However, the first shoot borne node, which was termed the coleoptile node (CN), only became distinguishable by day 4–5 (Fig. 1c–d) and produced true crown or nodal roots (NR) by day 7, when the second leaf was expanding (Fig. 1e–f).

The origin and type of roots in sorghum seedlings was simpler than for maize (Fig. 2). After germination, sorghum produced a single primary root and a coleoptile. By day 7, the coleoptile had produced two leaves, similar to maize, and the primary root had started to form lateral branches. In contrast to maize, however, no coleoptilar, nodal, or scutellar seminal roots had formed by day 7 (Fig. 2c).

Maize and sorghum differed in root system development during early growth (Exp2)

Species differences in root architecture in the seedling stage were maintained during early growth (Fig. 3). Within species, however, genotypic differences for root number and root length were generally not significant (Table 1), even though variation existed among individual plants within species. Therefore, the comparison of root architecture in Fig. 3 focuses on species differences, using photographs of representative plants.

In maize, nodal roots (NR) had already appeared by the time the 2nd leaf was fully expanded (Fig. 3a), which was consistent with results of Exp1. First order lateral root branches (BR) had also started to appear at this stage and were clearly visible by 3rd leaf fully expanded (Fig. 3b), but no 2nd order lateral branches were observed until at least the 6th leaf stage (Fig. 3e). In sorghum, the seminal root system, which consisted only of the primary root, was more vertically oriented than in maize. Lateral branches (BR) of PR were apparent by the 2nd leaf stage (Fig. 3f) and higher

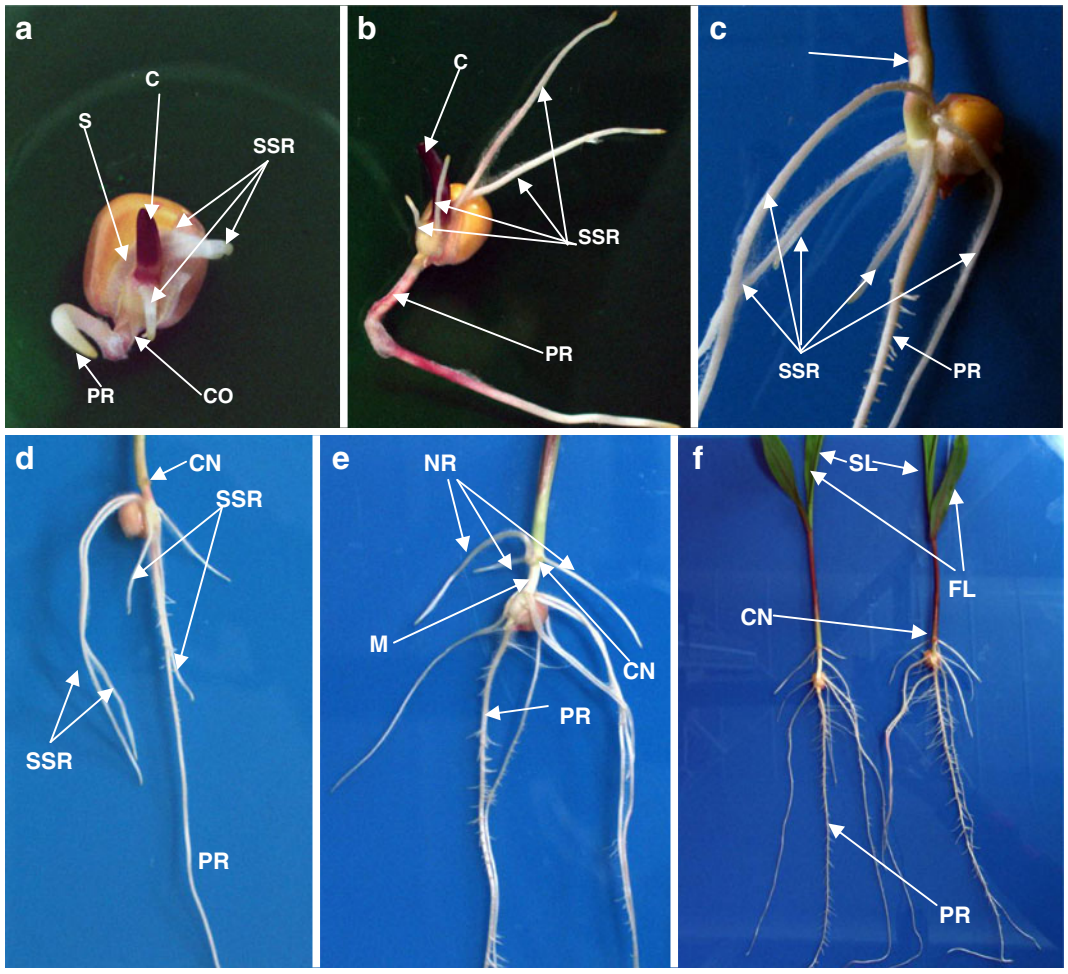

Fig. 1 Photographs of maize seed (**a**) 2 days, (**b**) 3 days, (**c**) 4 days, (**d**) 5 days, and (**e** and **f**) 7 days after germination. C, coleoptile; CN, coleoptile node; CO, coleorhiza; FL, first leaf; M, mesocotyl; NR, nodal root; PR, primary root; S, scutellum; SL, second leaf; SSR, scutellum seminal root. Photographs include 3343 and 3394

order lateral branches appeared with increasing plant growth (Fig. 3g–j). However, nodal roots (NR) only started to appear when 4–5 leaves had fully expanded. In both species, nodal roots appeared in pairs at every flush. The new flushes of nodal roots appeared at a similar rate to new leaves, and developed sequentially from lower shoot nodes upwards. Roots appearing from higher nodes had greater diameter and more vertical angle than those appearing from lower nodes.

The earlier appearance of nodal roots in maize, and the absence of scutellum seminal roots in sorghum, resulted in consistent differences in root system development between the two species during the period from leaf stage 2–6 (Fig. 3). Seminal root length of maize was around 5 times more than that of sorghum at the 2nd leaf stage (Fig. 4), and around 4 times that of sorghum at the 6th leaf stage (Table 1), because of the additional contribution of the SSR. Nodal root length at the 6th leaf stage was also more in maize than in sorghum (Table 1) and this reflected difference in plant size. The average root mass of maize at the 6th leaf stage (1.45 g plant^{-1}) was more than 3 times that of sorghum (0.45 g plant^{-1}) and similar differences were observed for leaf area and shoot mass (Table 1). Despite the differences in root length, both species had occupied most of the space in the root chamber at the 6th leaf stage (Fig. 3).

Genotypic differences in root system development were related to root angle (Exp2)

The two maize hybrids took 21 days on average from emergence to full expansion of leaf 6. They did not

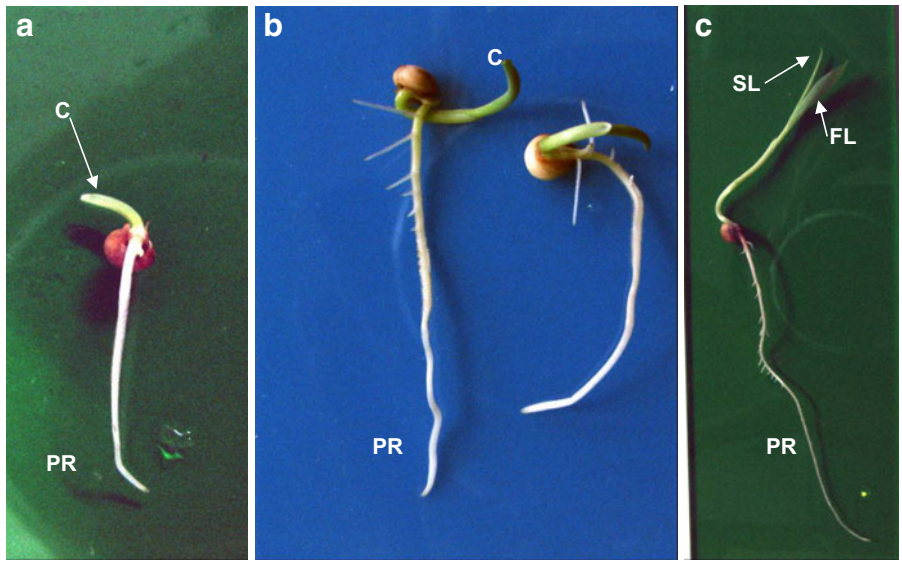

Fig. 2 Photographs of sorghum seed (**a**) 3 days, (**b**) 4 days, and (**c**) 7 days after germination. C, coleoptile; FL, first leaf; PR, primary root; SL, second leaf. Photographs include ATx642/RQL36 and ATx642/RQL12

differ significantly in any of the plant attributes measured at this stage, except for nodal root angle, which was significantly narrower for 3394 than for 3343 (Table 1). There was a tendency for 3394 to have lower linear root density than 3343 (0.0019 vs. 0.0029 g cm^{-1}) and this was consistent with its lower mean diameter of nodal roots (Table 1).

Sorghum hybrids took between 17 days (ATx642/RQL12) and 20 days (AQL39/RQL36) to reach the 6th leaf stage (Table 1). Genotypic differences in leaf

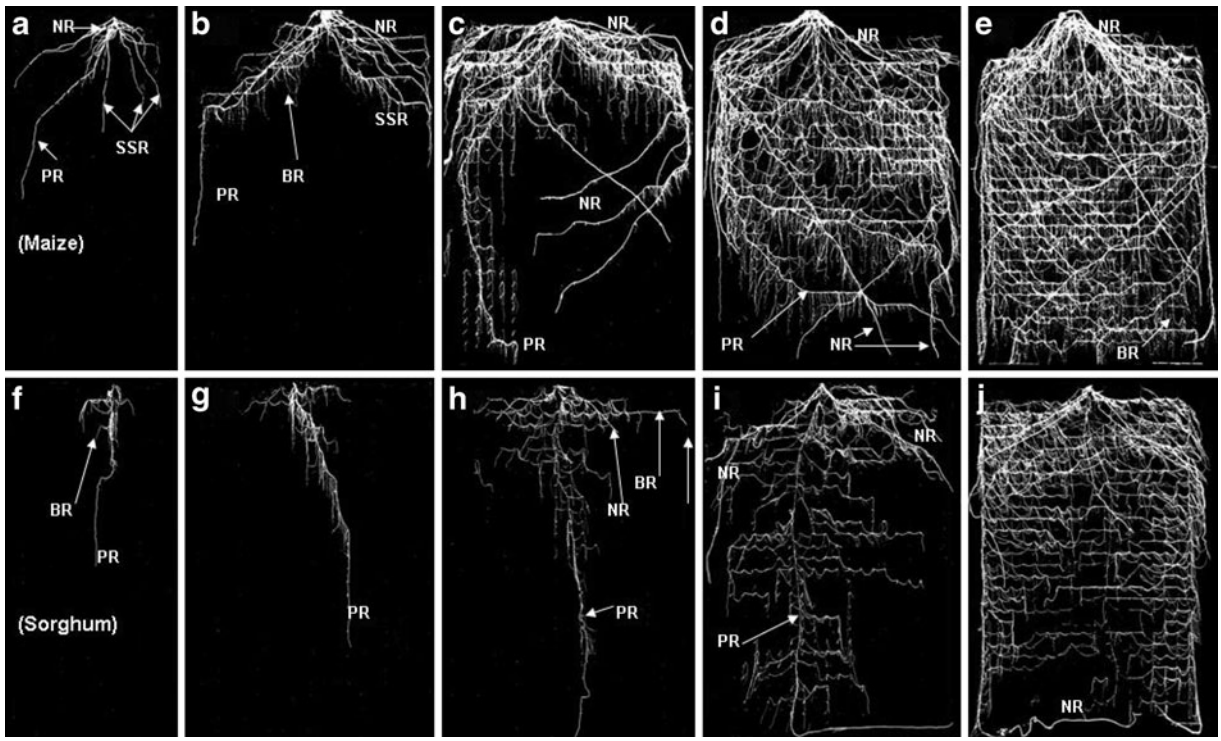

Fig. 3 Digital images of root systems of maize hybrid 3343 (**a**–**e**) and sorghum hybrid ATx642/RQL12 (**f**–**j**) at leaf stage 2 (**a**,**f**), 3 (**b**,**g**), 4 (**c**,**h**), 5 (**d**,**i**), and 6 (**e**,**j**). BR, branch root; NR, nodal root; PR, primary root; SSR, scutellum seminal root

Table 1 Shoot and root attributes of sorghum and maize hybrids measured at 6th leaf developmental stage in Exp2

	Sorghum					Maize		
	ATx642/ RQL12	ATx642/ RQL36	AQL39/ RQL12	AQL39/ RQL36	signif.	3343	3394	signif.
Days to harvest	17.0	18.7	19.3	20.3	***	21.0	21.5	ns
Shoot dry weight (g)	0.907	0.970	0.570	1.323	**	3.71	2.50	ns
Root dry weight (g)	0.287	0.407	0.257	0.867	*	1.63	1.27	ns
Root/Total biomass ratio	0.24	0.29	0.29	0.39	*	0.31	0.34	ns
Leaf area (cm^2 $plant^{-1}$)	237	269	180	382	*	833	631	ns
No of seminal roots	1	1	1	1	ns	3.5	3.5	ns
No of nodal roots	7.0	6.7	5.2	10.0	ns	9.0	12.0	ns
Nodal root angle 1st flush (º)	52.0	37.5	38.2	42.5	**	37.8	19.5	*
Mean nodal root diameter (mm)	1.3	1.5	1.2	1.6	*	2.3	1.6	ns
Total seminal root length (cm)	59.7	63.5	63.5	67.3	ns	317	242	ns
Total nodal root length (cm)	205	290	217	304	ns	384	561	ns
Linear root density (g cm^{-1})	0.0013	0.0018	0.0015	0.0027	**	0.0029	0.0019	ns

ns not significant

*$P<0.05$; **$P<0.01$; ***$P<0.001$

area, dry weight, and root length became apparent around the 6th leaf stage (Fig 4). The two RQL36 hybrids had the greatest total root length, and this was associated with greater root mass and larger root diameter. This was particularly the case for AQL39/RQL36, which had the greatest linear root density and, consistent with this, the greatest mean diameter of nodal roots (Table 1). The root angle measured on the primary flush of nodal roots differed significantly among sorghum hybrids, with ATx642/RQL12 having the widest angle (Table 1, Fig. 5), indicative of a more horizontal root system (Table 1).

Genotypic differences in root elongation rate were small (Exp3)

The average root elongation rate (RER) was around 2.6–2.7 cm d^{-1} for both sorghum and maize (Table 2). However, significant genotypic differences were observed for both species. For maize, the RER of 3394 (2.91 cm d^{-1}) was significantly greater than that of 3343 (2.56 cm d^{-1}). For sorghum, the RER of AQL39/RQL36 (2.80 cm d^{-1}) was significantly greater than that of ATx642/RQL36 (2.39 cm d^{-1}), and AQL39/RQL12 (2.51 cm d^{-1}), whereas ATx642/RQL12 (2.65 cm d^{-1}) was intermediate.

Discussion

Germination and origin of seminal and nodal roots in maize and sorghum

There remains some confusion over the classification of root types in sorghum and maize with various studies advocating differing approaches (Hochholdinger et al. 2004; Watt et al. 2007). Sorghum and maize, like other monocotyledons, have seminal roots that appear from the seed, and nodal roots that appear from the coleoptile node. Seed-originating seminal roots include the primary root, which originates from the coleorhiza, and, in maize, roots (SSR) that originate from the scutellum node inside the seed (Esau 1967; Hochholdinger et al. 2004). The scutellum originating roots in maize have initiated primordia in the seed (Esau 1967), whereas we did not observe this in seed sections of sorghum (data not shown). Interestingly, the *rtcs* (rootless concerning crown and seminal roots) mutant of maize does not produce any SSR either, although it also lacks nodal roots (Taramino et al. 2007). The lack of SSR in sorghum has some similarity with the phenotype of this single gene mutant.

It has been widely reported that seminal roots of maize can persist and remain functional throughout

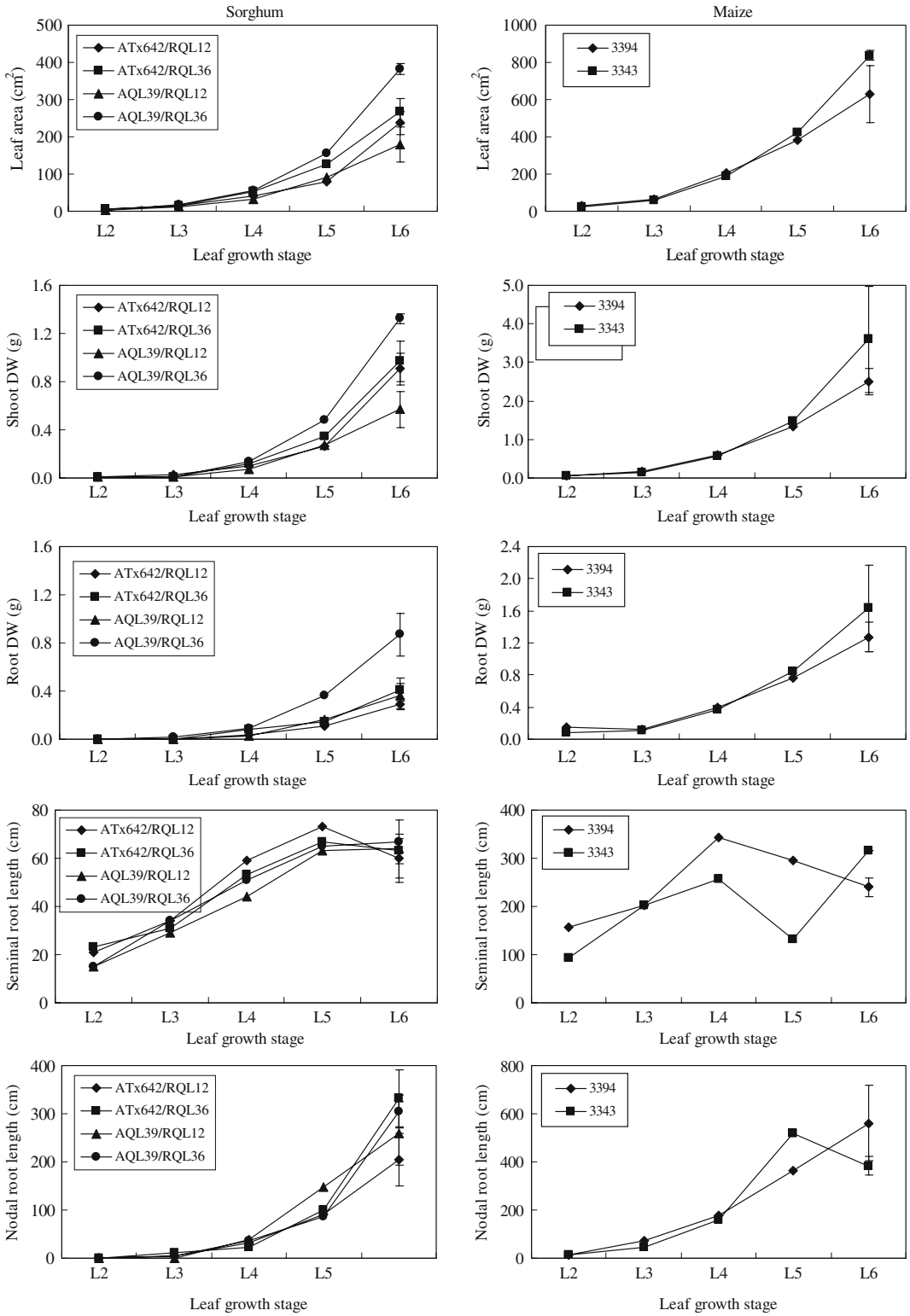

Fig. 4 Leaf area, shoot and root dry weight, and total length of seminal and nodal roots per plant versus stage of development from 2 (L2) to 6 (L6) fully expanded leaves on the main culm for sorghum and maize genotypes in Exp 2. Bars indicate standard error of means at L6

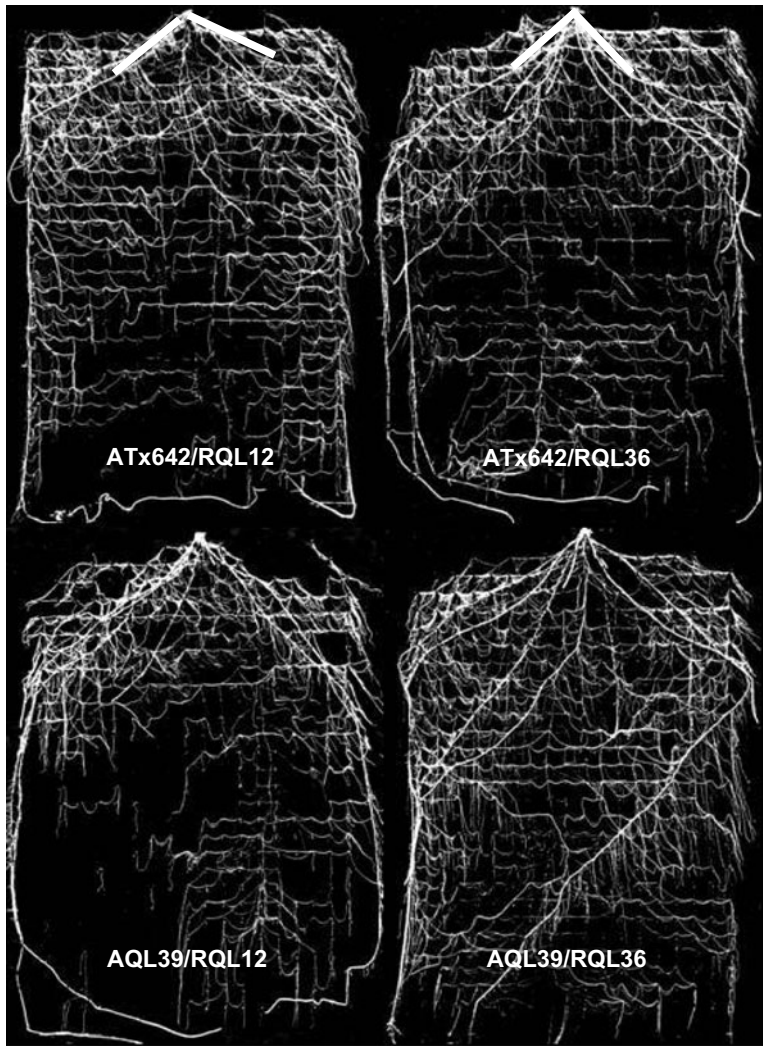

Fig. 5 Intact root systems of four contrasting sorghum genotypes at the 6 leaf stage in Exp 2. *Thick white lines* included for ATx642/RQL12 and ATx642/RQL36 highlight the angle for the flush of nodal roots

the life cycle of the plant (Kausch 1967; Kiesselbach 1949; Kozinka 1977; McCully and Canny 1988), although some authors have observed that the primary and other seminal roots die after the formation of the shoot-borne root system (Feldman 1994; Lawson and Hanway 1977). Reports on the presence of a scutellum are common for maize (Hochholdinger et al. 2004; Mollier and Pellerin 1999; Nieto-Stoleo et al. 2002), but not for sorghum (Aloni and Griffith 1991), even though the scutellum is an integral part of the sorghum seed (Dunstan et al. 1978). This likely reflects the absence of scutellum seminal roots in sorghum. In studies on wheat root systems that examined the origin, number, and arrangement of xylem tracheary elements (XTE), Watt et al. (2007) classified all major roots, except the primary seminal root, as nodal roots. They found that the anatomical arrangement of XTE for the roots originating from the scutellum was closer to that of the nodal roots originating from the coleoptile node than to the primary root originating from the coleorhiza. Hochholdinger et al. (2004) also found that roots initiated from different tissues during embryonic and post embryonic development can have similar anatomical structure. Hence, this suggests that apart from point of origin, there is little to distinguish seminal roots arising at the scutellar node from the later-formed nodal roots.

In addition to the absence of roots originating from the scutellum, an important difference in root system development of sorghum compared to maize was the later onset of coleoptile node roots. In sorghum these roots did not appear until 4–5 leaves had fully

Table 2 Average root elongation rate (RER, cm day^{-1}) for four sorghum and two maize hybrids. RER was estimated as the slope of the linear regression of rooting depth versus time from sowing in Exp3 (see text). The standard error (s.e.) of the slope and coefficient of determination (R^2) are also given

Hybrids	RER[a]	s.e.	R^2
Sorghum			
ATx642/RQL12	2.65abc	0.41	0.85***
ATx642/RQL36	2.39a	0.37	0.90***
AQL39/RQL12	2.51ab	0.23	0.97***
AQL39/RQL36	2.80c	0.35	0.90***
Maize			
3394	2.91c	0.30	0.93***
3343	2.56ab	0.32	0.93***

[a] entries followed by the same letter are not significantly different at $P<0.05$ based on analysis of covariance; ***, $P<0.001$

expanded, which was slightly later than the three fully expanded leaf stage reported by Blum et al. (1977a). Because of the later onset of nodal roots, sorghum relied on its seminal root system for longer than maize. As primary roots tend to grow more vertically than the scuttellar seminal roots and nodal roots, the sorghum root system had a more vertical orientation at the 3–4 leaf stage than maize (Fig. 3). In addition, nodal roots of maize at lower nodes tend to have a wider angle than those at higher nodes (Feldman 1994). Hence, the later appearance of nodal roots in sorghum may exacerbate the vertical architecture of its root system. These species differences could represent evolutionary differences, where the early nodal root development of maize favors anchoring of the plant, particularly in wet soil, whereas the vertical primary root growth of sorghum favours exploration of water at depth, which potentially enhances survival during drought (Kato et al. 2006; Manschadi et al. 2006; Oyanagi 1994).

Root angle as a phenotypic screen for root architecture

The branching angle of the first flush of nodal roots was the only parameter that showed significant genotypic differences in both maize and sorghum (Table 1). The growth angles of roots are genetically defined (Oyanagi et al. 1993; Wang et al. 1995) and QTL's associated with root angle have been identified in maize and rice (*Oryza sativa* L.) (Norton and Price 2009; Omari and Mano 2007). Genotypic differences in root angle have been reported for seminal roots in wheat (Manschadi et al. 2008; Oyanagi 1994) and barley (Hargreaves et al. 2009) and nodal roots in rice (Kato et al. 2006; Tsuji et al. 2005) and maize (Giuliani et al. 2005; Omari and Mano 2007). A narrow growth angle has been related to greater root diameter in both sorghum (Tsuji et al. 2005) and rice (Kato et al. 2006) and thicker roots in turn can have greater RER (Cahn et al. 1989). Our results showed similar associations between root angle, root thickness, and RER. Maize hybrid 3394 had a significantly narrower root angle than 3343, and this translated into a significantly greater RER, although the hybrids did not differ significantly in basal root diameter or linear root density (Table 1). For sorghum, drought susceptible AQL39/RQL36 tended to have a narrower root angle, thicker diameter and greater linear root density and RER than drought tolerant ATx642/RQL12 (Table 1, Fig. 5). The drought susceptible sorghum hybrid thus had root attributes expected to be associated with a drought adapted phenotype. A potential explanation for this discrepancy is that the drought adaptation classification for sorghum hybrids was based on differences in staygreen, which for these hybrids was likely related to differences in plant size and thus in demand for water (van Oosterom et al. 2008), rather than to differences in root architecture affecting access to water (supply). Plant size and root architecture thus represent two contrasting drought adaptation mechanisms, and the results for sorghum imply that these mechanisms could potentially operate independently, as has been observed in rice (Price et al. 2002).

Differences in root angle can affect the spatial pattern of water extraction (Manschadi et al. 2006; Hammer et al. 2009). A narrow root angle may affect the vertical distribution of roots through deeper rooting, which can improve water capture at depth and thus positively affect drought adaptation (Hammer et al. 2009; Kato et al. 2006; Oyanagi 1994). In contrast, a wider angle favours better lateral access, which could increase water extraction in wide or skip row dryland farming systems (Whish et al. 2005). The importance of root angle thus depends on the environmental and management conditions under which a crop is grown.

In contrast to root angle, genotypic differences in root length or root mass were either non significant,

or reflected differences in plant size (Table 1, Fig. 4), which was consistent with previous results reported for sorghum (Blum et al. 1977b). This makes these traits unsuitable as selection criteria for root architecture. The existence of significant genotypic differences in root angle in a range of cereals, its apparent independence of plant size, its importance to root architecture, and its potential implications for water capture, make root angle a useful selection trait for breeding programs targeting adaptation to specific environmental and management conditions, although its role in drought adaptation may depend on its interaction with other traits affecting drought adaptation of the crop.

Implications for large-scale screening for root architecture

A major bottleneck to using root traits in breeding programs has been the lack of a high throughput screening system. Particularly for sorghum, screening of seminal roots has limited value, as they do not constitute the major part of the mature root system (Blum and Ritchie 1984; Salih et al. 1999; Tsuji et al. 2005). In our experiments, sorghum plants produced only one primary root and root systems consisted predominantly of nodal roots. Further, the observation that nodal roots only appeared at the 4–5 leaf stage in sorghum indicated that screening for root angle can only be done from that stage onwards. The size of the seminal root system and of the shoot at this stage (Fig. 4) and the time it takes the plant to reach this stage thus place restrictions on the size of the chamber and the growing medium that could be used for screening experiments.

The size of the root system at the 6th leaf stage (Fig. 4) requires chambers for screening of root angle to be at least 50 cm deep. In addition, sorghum plants need to grow for nearly 3 weeks before nodal roots start to appear. Gel chambers have been successfully used to study root architecture in wheat (Bengough et al. 2004; Manschadi et al. 2008) and barley (Hargreaves et al. 2009) and blotting paper pouches have been used for maize (Hochholdinger et al. 2004; Hund et al. 2009; Trachsel et al. 2009). However, because seminal roots were measured in wheat and barley, and scutellar and nodal roots appear early in maize, the size of the chambers used and the duration of the experiment were both much less than that required for sorghum. In our study, preliminary experiments using large gel chambers failed, because of difficulties in maintaining a consistent density of gel, meeting the nutrient requirements of the seedling for the extended duration of the experiment, and an increased problem with contamination, an issue also identified by Hargreaves et al. (2009). Similarly, blotting paper pouches are unlikely to work for sorghum, as autotrophic growth only lasts for approximately 10 days in maize (Hund et al. 2009) and is unlikely to be longer in sorghum. Therefore, small chambers of at least 50 cm deep and a few millimeters thick, filled with soil, appear to be the preferred apparatus for large scale phenotypic screening of root angle in sorghum, as these allow plant growth till the 6th leaf stage, and in situ scanning of roots for their angle.

Based on these specifications, small prototype chambers (50×45×0.3 cm) have been tested in genotype screening (Singh et al. 2008) and this is now the focus of current research on genotypic variation. Manual assembly and filling of 100 chambers takes a few days and in situ scanning of the root system (for root angle) is limited only by the speed of the scanner. The system thus provides a moderately rapid 2D screening for root angle, similar in throughput to 2D gel chambers (Hargreaves et al. 2009). Compared with X-ray tomography (Hargreaves et al. 2009) or MRI-PET (magnetic resonance imaging–positron emission tomography) scans (Jahnke et al. 2009), which provide non-destructive 3D scans of roots in large containers, scanning of gel chambers or soil-filled chambers is considerably cheaper and has a higher throughput (Gregory et al. 2009). Moreover, results of Hargreaves et al. (2009) showed that observation of root angle of barley in 2D gel chambers were representative of the 3D angular root spread measured with X-rays. For screening of root angle in sorghum, soil filled chambers of at least 50×45 cm and a few millimeters thick thus compare favourably, in terms of costs and throughput, with available methodologies for other crops.

Conclusion

Maize and sorghum differed in root development at the seedling stage for both the number of seminal roots and the timing of nodal root appearance. Sorghum produced only one primary root from the

seed and coleoptile nodal roots emerged at the 4th–5th leaf stage, whereas maize produced 3–7 primary and scutellum roots from the seed and coleoptile nodal roots emerged at the 2nd leaf stage. Our results indicated that characterization of nodal roots, and in particular their angle, could be considered for large scale screening for root architecture in breeding populations, as these traits are easy and inexpensive to measure. Because of the relatively late appearance of nodal roots in sorghum, such screening can only be performed when approximately 6 leaves have fully expanded and will require a chamber at least 50×45 cm large and a few millimeters thick. Large scale screening of young plants needs to be accompanied by testing of mature plants to verify whether differences in root attributes of young plants do translate into differences in water extraction patterns of mature plants.

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