

How Sorghum Root Traits can Contribute to Cereal Yield Increase

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Abstract

In recent decades the effects of climate change became more visible and the problems it causes for agricultural production and yield maintenance. Future crops need to be higher yielding than today, but at the same time more resilient to drought and increased temperatures, especially in drought-prone regions with erratic precipitation. Sorghum, more heat and drought tolerant than maize, presents an interesting candidate for potential genetic material to provide this increased resilience, containing traits and the underlying genetic loci conferring better performance. Compared to the above-ground tissues, root systems are less investigated, but an improvement in this “hidden half” also improves yield. Due to their close relationship, findings in sorghum may be easily incorporated into maize breeding programs. In this chapter we will review recent literature on sorghum and other cereal root system improvements and provide unpublished data on the natural variation of sorghum root development.

Keywords: Sorghum, root, root hair, natural variation, genomic diversity

1. Introduction

The domestication of Sorghum (*Sorghum bicolor* (L.) Moench) occurred in the region of present-day Sudan approximately 10,000 years ago. It diffused to diverse climates and regions across Africa, India, the Middle East, and Asia between 8,000–1,500 years ago [1, 2]. More recently, diffusion occurred to more temperate zones of northern China and the introduction to North America expanding the range of sorghum cultivation even further [1, 3]. *Sorghum bicolor*, the 5th most important cereal in the world behind maize, rice, wheat, and barley, is grown in both subsistence and commercial agriculture. It is a major crop in the semiarid regions and a dietary staple for more than 500 million people predominantly in Sub-Saharan Africa and South Africa [4–6]. Sorghum, a C4 grass, is cultivated for production of grain, forage, sugar / syrup, brewing, lignocellulosic biomass, and bioethanol [7–9]. Climate change threatens the agricultural production and food security in semiarid regions increasing the importance of drought-tolerant crops. Although grain yield gains for maize have been higher than for sorghum, especially under rain-fed management in high water-holding capacity soils [10], sorghum has a higher water use efficiency compared with maize, when grown under optimal growing conditions [11]. Sub-Saharan Africa and South Asia are predicted to have the greatest decline in agricultural productivity due to a significant risk of rising temperatures [12]. These geographical regions overlap with areas with drought and erratic rainfall, where sorghum is already grown as a major staple food. There,

sorghum is an important crop for subsistence farmers in these regions due to higher yields compared other cereals in drought and low perceptions regions, which make these areas unsuitable for maize and rice [13, 14]. However, grain yield of sorghum is likely to be affected by post-anthesis drought stress in rainfed farming systems of northeastern Australia [15], India's western-central monsoon region [16], the southern USA [17], and sub-Saharan Africa [18–20]. Selection for stay-green in elite sorghum hybrids should have the potential to increase yield, profitability, and sustainability for farmers in rainfed environments worldwide, without greater yield penalties during wetter years.

Sorghum evolved after splitting from the shared ancestors with rice in Africa 50–70 million years ago, but diffusion into other regions and the widespread cultivation led to high natural genetic diversity within sorghum nowadays [1], which has resulted in distinct phenotypic variance defined by their floral architecture and seed characteristics [6, 21]. Sorghum is a diploid C4 grass with 10 chromosomes and a genome of approximately 800 Mbp [22, 23]. A first reference genome was reported in 2009 [8]. The reference genome of sorghum is derived from the inbred 'BTx623,' a genotype with reduced height and early maturation, which is primarily used for production of grain. The phenotype of this reference genotype is very distinct from the tall, late maturing sorghums, which are usually grown for sugars or high biomass yield [1]. Commercial production systems in Argentina, Australia, Brazil, Mexico or USA utilize sorghum hybrids. However, subsistence agriculture mainly plants sorghum inbred lines for their livelihood. The preference of both consumers and regulators for non-GM sorghum has focused significantly on identifying and utilizing the natural genetic variation of sorghum to improve yield and quality. Currently, Sorghum breeding focusses on tackling abiotic and biotic stresses such as drought, acid soils, and insect and fungal pests [4]. The genetic resources that are largely created by public research are important to understand crop physiology to improve crop performance and production. It is aided by genome-wide map of SNP variation that will accelerate marker-assisted breeding. The adaptability and stress tolerance found in sorghum accessions allows to study the genotype–phenotype relationship as well as dissect genotype-by-environment (G x E x M) interactions for complex, quantitative traits [24] permitting future insights in drought tolerance and thereby mitigating the impacts of climate change. Especially, the exploration of the unknown and unexplored genetic potential taking advantage for the improvement of other cereals, especially maize.

The origin in Africa, distribution to other ecosystems, and agricultural practices is reflected in the phenotypic variation [21] ranging from traditional varieties across Africa and Asia to modern germplasm in China, Australia, and the Americas. This provides a wide variance of morphological and physiological traits for crop improvement [3, 7, 25]. Rainy periods are long and erratic in parts of West Africa, and subsequently, open panicle guinea types are preferred to reduce yield penalties such as grain mold and insect damage. In contrast, other parts of South and East Africa, where rainy seasons are relatively short and predictable, dense panicle kafir and durra types are preferred to increase grain yield per plant [4]. Further selection has occurred in the United States in the last 150 years as temperate and tropical sorghum from Africa and Asia has been bred for commercial agriculture [26].

While research on climate change impact on sorghum is limited, the importance of its root system has been highlighted. Modelling studies have shown that sorghum root systems have a relative adaptive advantage over maize in water-limited conditions [27]. The differences between maize and sorghum root system might facilitate adaptation to drought-prone regions with erratic precipitation. 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 [28]. After germination, sorghum

produced a single primary root and a coleoptile, by day 7 the two leaves stage was reached and the primary root had started to form lateral branches. In contrast to maize, no nodal or seminal roots had formed by day 7 (see also **Figure 1**). Sorghum produced only one primary root from seed and nodal roots emerged at the 4th–5th leaf stage, whereas maize produced 3–7 roots from the seed and nodal roots emerged at the 2nd leaf stage [28]. The differences in root development and the adaptation to different environmental and agricultural practices of sorghum root

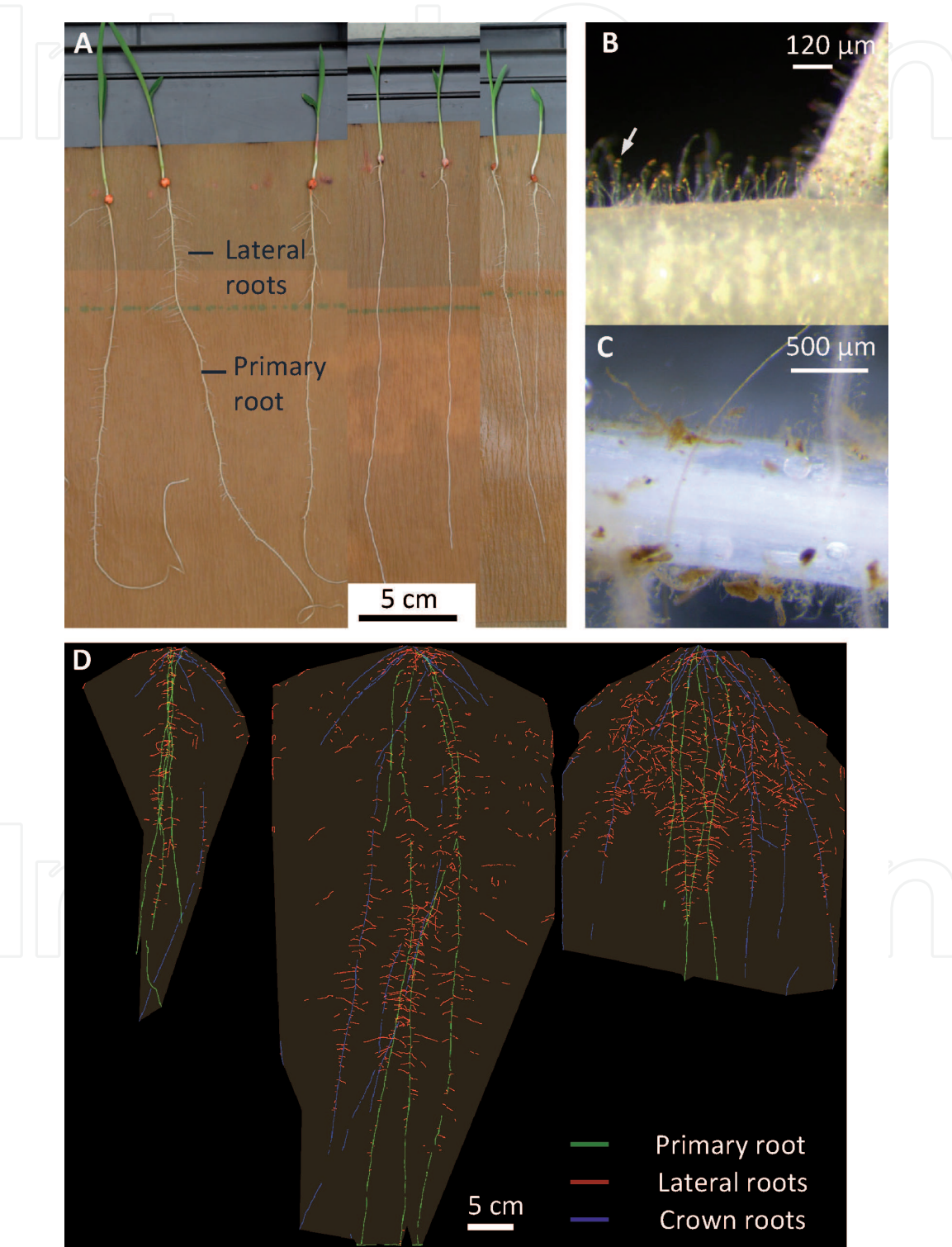


Figure 1.
The root system of *Sorghum bicolor*. Depicted are three european sorghum genotypes either germinated and grown in filterpaper for 14 (A) or 10 days (B) and for 21 (C) or 14 days in soil (D). Root systems and root hair formation of the varieties ‘WLo8–713’ (A,B,D) and ‘Zerberus’ (A,C,D), and ‘SOR19’ (A, D) are shown from left to right. The arrow in (B) highlights sorgoleone excretion at root hair tips. In (D) the Maximum Intensity Projections of traced roots grown in rhizotrons of 4 plants per genotype are visible.

systems might explain the better performance of sorghum in drought-prone regions with erratic precipitation when compared with maize. Increased access to water can be achieved either by better water acquisition from the soil exploring an increased soil volume, which could be achieved by deeper rooting or greater lateral spread [29, 30]. A relationship between drought adaptation and nodal root angle was reported, which further supports the role of below-ground biomass traits in sorghum production under water stress [31]. Furthermore, QTLs were mapped for nodal root angle in sorghum at the 6-leaf stage and evaluated the relevance of the trait for improving drought adaptation via marker-assisted selection. All four nodal root angle QTLs in sorghum identified co-located with previously identified QTLs for stay-green loci [31]. The grain yield benefit of the stay-green phenotype under drought was found to be a result of reduced vegetative biomass and water uptake during the pre-flowering growth stages [32]. Under artificial conditions, sorghum root length during the seedling stage was found to be a major factor in drought tolerance [33].

The parasitic plants, *Striga asiatica* and *Striga hermonthica*, cause serious yield penalties in subsistence and commercial sorghum production. *Striga* is an obligate root parasite, which seeds will not germinate unless it receives a chemical signal from a potential host plant [34]. Chemicals identified in sorghum root exudates promote seed germination of *Striga*, the most potent are the strigolactones, a class of related compounds used by most terrestrial plants as hormones to regulate shoot and lateral root development [35, 36], and symbiotic colonization by arbuscular mycorrhizal fungi (AMF) [37]. Sorghum produces several strigolactones and exudes them from its root hairs, particularly under conditions of phosphorous and nitrogen limitations, promoting mycorrhizal association [38]. Colonization with AM fungi greatly improve the performance of sorghum in low-nutrient and drought environments [39]. *Striga* seems to utilize the signaling to detect its proximity to sorghum roots, so germination at the right time and place will increase the chances of infestation and completing its life cycle. The breeding strategy of *Striga*-tolerant lines included the introgression of lines that exude less of the *Striga*-promoting strigolactones, reducing yield penalties through *Striga*.

Root growth is impeded by aluminum, the third most abundant element in the Earth's crust. A major physiological mechanism facilitating plant aluminum tolerance is aluminum exclusion from root apices based on organic acid release forming stable, nontoxic Al^{3+} -complexes in the rhizosphere. Quantitative RT-PCR analysis showed that the responsible gene (*AltSB*) was expressed only in roots of the aluminum-tolerant near isogenic lines and was induced by aluminum. Its expression was highest in the first centimeter of the root [40]. The aluminum and *Striga*-tolerance are rare examples of adaptive root traits being part of breeding programs. Sorgoleone has similar potential due to its allelopathic properties and facilitation of arbuscular mycorrhiza. Sorghum performance on tropical soils is the result of adaptations to multiple stress conditions, including tolerance to aluminum toxicity, efficient acquisition of drought conditions increase the impedance of soils. Sorgoleone is a major component of sorghum root exudates (see also **Figure 1**). It composes from 76 to 99% of the total exudates from sorghum root hairs [41], and is one of the most studied allelopathic chemicals [42]. Phosphorus (P) is immobilized in aluminum and iron complexes due to low pH in tropical soils (reviewed in [42–44]). Several root system properties can facilitate P uptake efficiency by responses of root system morphology and architecture [45], modulation of P transporters, organic acid exudation, phosphatase secretion, and association with arbuscular mycorrhizal fungi (AMF) (e.g. [46]). The utilization of sorgoleone in breeding programs could facilitate crop production in drought-prone regions and mitigate the effects of climate change. Sorgoleone synthesis is constitutive and compartmentalized within root hairs, which can accumulate up to 20 μg of exudate

per milligram of root dry weight [47, 48]. Attracting AMF for an increased P uptake efficiency is one opportunity, root hairs offer another opportunity to mitigate the effects of climate change such as drought and low precipitation as at low P concentration in tropical soils, root exudation and subsequent mycorrhizal colonization will increase the phosphorous uptake efficiency [44, 48–51]. Furthermore, root hairs play an important role in the uptake of soil phosphorous and water [52–55] as they facilitate acquisition of immobile nutrients such as phosphorous and potassium through increased soil exploration. Acquiring nutrients and water from tropical soils requires a root system that explores the soil volume to deliver these water and nutrients. Soil exploration is often impeded by increased soil strength [43, 56, 57] but a study suggests that root hairs can provide anchorage force required to penetrate tropical soils [56] concluding that root hairs provide anchorage for individual maize root tips and that could provide anchorage for root penetration. Nevertheless, the degree of anchorage provided by root hairs will depend substantially on root hairs and mucilage production. The phenotypic and underlying genotypic potential of sorghum, especially the less studied root traits such as root hairs, has a great potential for breeding as breeding is a necessity in production of new, ideally improved varieties. It requires traits-of-interest with proven effects and phenotypic variation, ideally based on genetic diversity in a known population [57, 58]. To what degree diversity in root phenotypes can be expected will be presented and discussed in the following sections.

2. Results & discussion

To exemplify what degree of variation can be expected by sorghum varieties, a set of European sorghum lines was grown under sterile conditions in filter paper and in soil-filled rhizotrons. Grown in filter paper, the primary root and its lateral roots were identified easily, while no seminal roots were observed (**Figure 1**). In agreement with [28] 14 and 21 days after sowing (DAS), no seminal roots were observed in any of the varieties grown, while a varying number of crown roots was found. All root types of all the tested sorghum varieties did have root hairs and all those hairs were excreting sorgoleone, visible as droplets on each root hair tip. On soil-grown roots no sorgoleone was observed, but that might have been absorbed by the surrounding soil or washed away during the washing procedures at harvest.

2.1 Diverse set of sorghum genotypes, but little aboveground diversity

30 diverse sorghum genotypes, selected for variation in origin and breeding status, including physiological traits such as drought tolerance, and flowering time, (summarized in **Table 1**) were grown in soil-filled rhizotrons and their roots and shoots non-invasively phenotyped over three weeks. Although genotypic variation was large for most traits, the mean shoot height over all genotypes followed a linear increase (**Figure 2**) and its variation was stable in the last week of growth. Both, shoot height as well as shoot dry weight had a variation of $\sim 2x$ and $\sim 4x$, meaning the largest genotype had a dry weight or shoot height twice or four times as large as the smallest genotype. At harvest, 21 DAS, the most contrasting lines had 35 cm compared to 60 cm high shoots and 0.23 g compared to 0.82 g shoot dry matter. Among the varieties with largest shoot height were ‘Mace Da Kunya’, ‘SC35’, and ‘Mota Maradi’, landraces described either as drought tolerant, post-flowering or pre-flowering drought tolerant, respectively. The shortest three varieties were ‘Tx430’, ‘Tx631’, and ‘Tx436’, all American feed-grade hybrids. Genotypes with large shoot height tended to also have higher shoot dry mass compared to genotypes with shorter

ID	Pedigree	Description	Origin
1	T × 430	Feed-grade hybrid pollinator-parent	USA
2	T × 2752	Feed-grade hybrid seed-parent	USA
3	T × 631	Food-grade hybrid seed-parent	USA
4	T × ARG1	Food-grade hybrid seed-parent	USA
5	T × 436	Food-grade hybrid pollinator-parent	USA
6	B N223	Food-grade hybrid seed-parent	Niger
7	SC599	Post-flowering drought tolerant accession	USA
8	SC35	Post-flowering drought tolerant accession	USA
9	Kuyuma	Improved, open pollinated variety	Zambia
10	Sepon82	Improved, open pollinated variety	Niger
11	SK 5912 Short Kaura	Improved, open pollinated variety	Nigeria
12	Ajabsido	Drought tolerant landrace	Sudan
13	CE-151-262-A1	Improved, open pollinated variety	Senegal
14	CSM-63	Drought tolerant landrace	Mali
15	Mota Maradi	Pre-flowering drought tolerant landrace	Niger
16	Koro Kollo	Pre-flowering drought tolerant landrace	Sudan
17	Feterita Gishesh	Pre-flowering drought tolerant landrace	Sudan
18	Segeolane	Pre-flowering drought tolerant landrace	Botswana
19	PI609567	Post-flowering drought tolerant accession	Mali
20	MR732	Elite, food-grade, hybrid pollinator-parent	Niger
21	Wassa	Improved, open pollinated variety	Mali
22	Seguetana	Improved, open pollinated variety	Mali
23	El Mota - S241	Pre-flowering drought tolerant landrace	Niger
24	Honey Drip	Sweet-stem sorghum	USA
25	Theis	Sweet-stem sorghum	USA
26	Framida	Improved, Striga-resistant variety	Burkina Faso
27	ICSV1049	Improved, Striga-resistant variety	Burkina Faso
28	Sariaso 14	Improved, Striga-resistant variety	Burkina Faso
29	Grinkan	Improved, open pollinated variety	Mali
30	Mace Da Kunya	Drought tolerant landrace	Niger

Table 1. Commercial sorghum parent lines and accessions. Sorghum bicolor lines selected for whole genome sequencing including diverse varieties from Africa, Striga-resistant lines from West Africa, and elite sorghum parent lines.

shoots. A higher variation in shoot biomass compared to shoot height implicated additional factors influencing the first independent of the latter, such as leaf number, width and thickness. Given the highly diverse origin of these selected genotypes (**Table 1**) a high phenotypic variation above- and below ground was expected.

2.2 Diverse set of sorghum genotypes with much higher belowground diversity

In contrast to the relatively small above ground variation in the rhizotron-grown sorghum lines, root dry matter varied much more after three weeks of

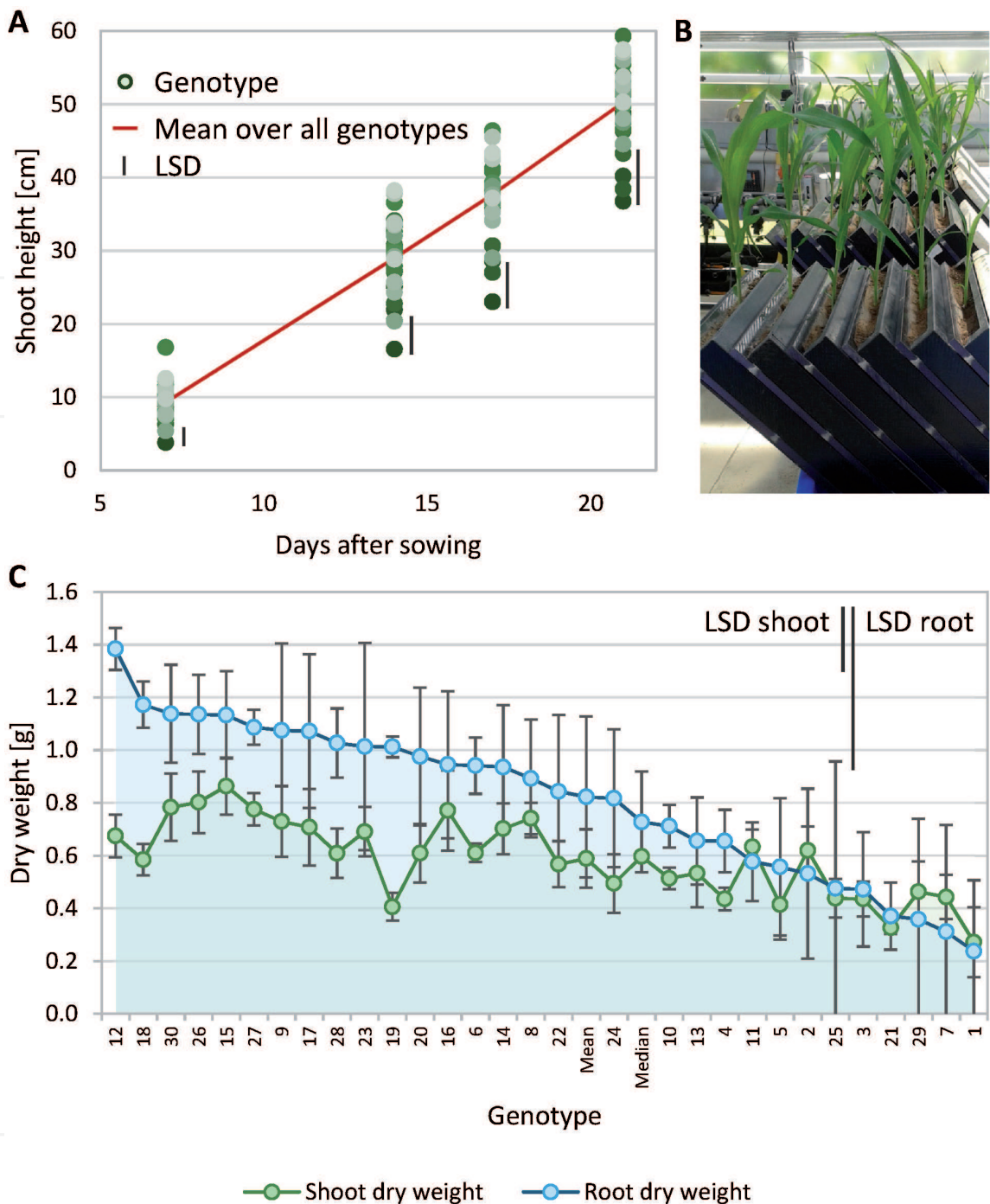


Figure 2. Variation in growth of 30 *Sorghum bicolor* genotypes. Shoot height of 30 sorghum genotypes over 3 weeks grown in soil-filled rhizotrons are shown (A). Single genotype values are means over 4 replicates. At harvest, 21 DAS (example image in B), root and shoot dry weight was determined (C) and shown per genotypes as mean ($n = 4$) $\pm$ SE. Per timepoint a one-way ANOVA Least Significant Difference (LSD) is depicted. Detailed information on genotypes can be found in Table 1.

growth- almost 7x between the most extreme genotypes (**Figure 2**). At harvest the root dry weight varied between 0.22 g and 1.4 g. Again, the three largest root biomass varieties were observed as drought tolerant landraces ('Ajabsido', 'Segeolane', 'Mace Da Kunya'), while improved and hybrid varieties had lower root biomass ('Tx430', 'SC599', 'Wassa'). A similar wide range of variation (6-7x) was found for root length of all separated types- the primary root, nodal roots, and lateral roots (**Figure 3**), but it changed over time. One week after sowing the first emerging primary root showed the highest length and variation while crown and lateral roots were almost not detected. Primary root length reached a plateau between 14 and 17 DAS, both due to the physical rhizotron constraints and the

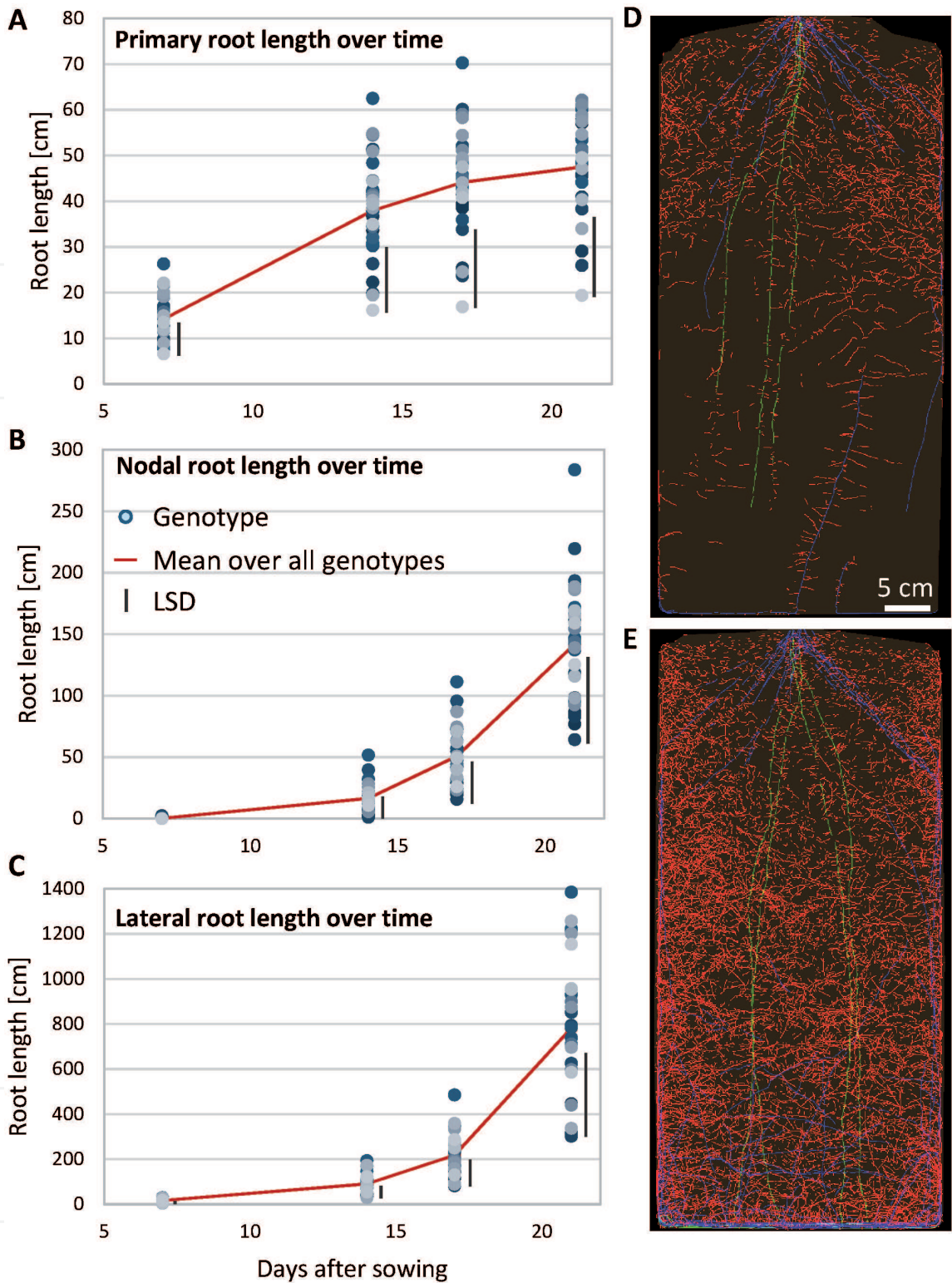


Figure 3. Variation in root growth of 30 *Sorghum bicolor* genotypes. Root length of 30 sorghum genotypes grown for 3 weeks in soil-filled rhizotrones are shown. Single genotype values are means over 4 replicates. Visible and traced primary root (PR) length (A), nodal root (NR) length (B), and lateral root (LR) length (C) are depicted. Maximum intensity projections of all replicates per genotype are shown for the smallest LR and NR length (D, genotype 'SC599') and the largest LR and NR length (E, genotype 'Mota Maradi'). Coloured lines represent traced PR (green), NR (blue), and LRs (red). Per time point a one-way ANOVA Least Significant Difference (LSD) is depicted. Detailed information on genotypes can be found in **Table 1**.

limited number of one primary root per plant. Two weeks after sowing, nodal root length varied from not detected to close to primary root length (50 cm), and just three days later their length doubled, and more than doubled again at harvest, 21 DAS. Lateral root length showed an even stronger increase in length over

time, the genotype with the longest LRs had 1,400 cm LR length at harvest, while the most contrasting genotype on the other end had only 250 cm LR length. With increase in NR and LR length over time, their variation among the tested genotypes also increased both in absolute and relative values. Although the genotypic ranking per investigated root type varied slightly, also over time, a general trend of stable ranking became visible. Since these plants were grown without nutritional, water, light, or biological stress this expresses their genetic potential to either form rather small or large root systems, often also with higher numbers of main axis. In all three root types, 'Tx436', a food-grade hybrid pollinator parent, and 'SC599' (**Figure 3D**), a post-flowering drought tolerant accession, were among the lowest ranking genotypes. Among the largest root systems were 'Mota Maradi' (**Figure 3E**), a pre-flowering drought tolerant landrace, and 'SK5912' and short 'Kaura', an improved open pollinated variety. Thus, previously drought tolerant described varieties did not show comparable root system developments in contrast to their early shoot development.

To gain more detailed knowledge about root morphology of these 30 sorghum genotypes, microscopic analyses were performed. Per root type (PR, NR, LRs) root diameter, root hair length, and root hair density were measured (**Figure 4**). When root hair density was plotted against root hair length per root type, a dependency became visible: roughly the more root hairs the longer they were (**Figure 4A**). All root types except for nodal roots showed significant correlations of root hair length and density. On all root types the genotypes 'Tx430', 'Tx631', 'Tx436', and 'Mace Da Kunya' formed the shortest and fewest root hairs. As root hairs are known to be instrumental for water and nutrient uptake [59] it is surprising to find 'Mace Da Kunya' in this list as it was also producing high root and shoot biomass. It should be noted that without nutrient and water limitation short and fewer root hairs were shown to be sufficient for plant growth [60, 61]. The longest and most root hairs were formed on roots of 'Segeolane', 'MR732', and 'Mota Maradi'. Since the latter, a pre-flowering drought tolerant landrace, also had the largest root system, it overall has the highest root surface area leading to the most soil contact for water and nutrient uptake. Like 'Mota Maradi', the genotypes with most root hairs also have the potential to excrete more sorgoleone into their soil environment compared to varieties with smaller root systems and fewer root hairs. Nodal roots had longer root hairs compared to all other root types, followed by primary roots, but their lateral roots did not differ from each other. Overall these soil-grown roots did produce many, but short root hairs of ~150 µm length; similar ranges of root hair formation have been reported for soil-grown rice varieties [60]. On the other hand, field-grown barley genotypes were reported to form longer root hairs from 400 µm [62] up to 700 µm [61]. Root hair formation in these studies varied with environmental conditions, be it nutrient or water supply, or other soil properties, therefore it is likely that sorghum root hairs could be longer in less optimal conditions than the one they were grown in here. Studies on rice root type-dependent root hair formation also showed a high dependency on the growth media used [63, 64].

The rhizotron-grown 30 genotypes showed root type-specific separation of root diameters (**Figure 4B**). For every genotype, nodal roots were not only thicker than primary roots, they did separate clearly with PRs ranging from ~300–550 µm and NRs from ~700–1,050 µm thickness. In contrast, their lateral roots had similar diameters, and both main roots (NR & PR) had 'thin' and 'thick' lateral roots, the first with ~100 µm diameter and little variation, the latter with higher variation from ~150–300 µm. In rice, distinct classes of lateral roots, S-type (thin) and L-type (thick) have been identified that are distinguishable by their diameter, but also branching ability [65, 66]. Recently those LR types have also been indicated to have

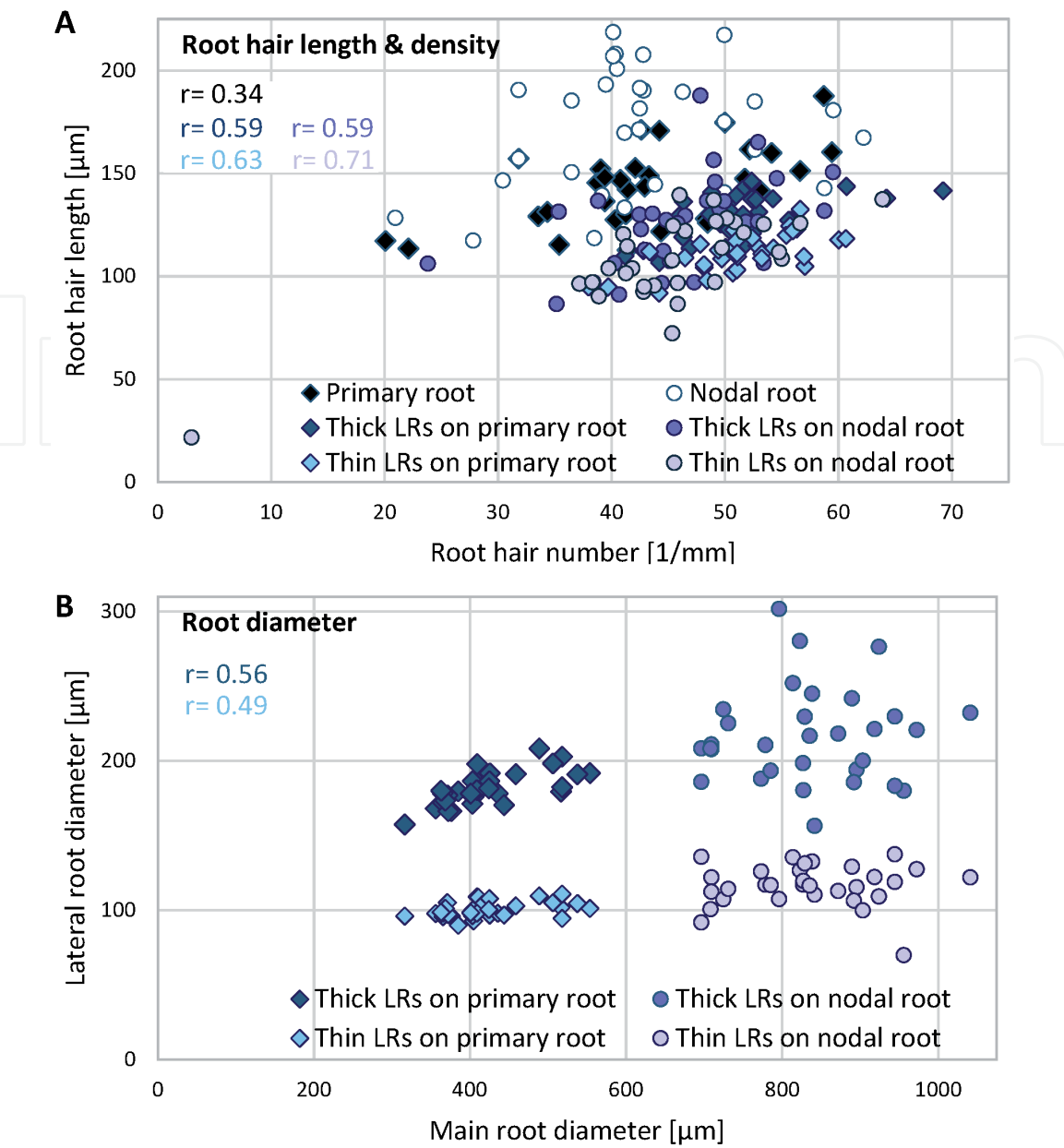


Figure 4. Root morphology of 30 *Sorghum bicolor* genotypes. Root morphological traits of 30 sorghum genotypes grown over 3 weeks in soil-filled rhizotrones were determined using a stereomicroscope and analysed with the software Image J. Each point represents a single genotype value which is a mean over 4 replicates. Shown are root hair length depending on root hair density per root type (A) and lateral root (LR) diameter depending on their main root diameter (B). Significant linear correlations are depicted. Detailed information on the genotypes can be found in **Table 1**.

different functions in water and nutrient uptake and transport [67, 68]. If these different diameters do also indicate different LR functions in *Sorghum* would be interesting to investigate in future experiments, especially with resource limited conditions. Interestingly, while the primary root diameter did significantly correlate with the diameter of its lateral roots, this was not found for nodal roots and nodal root lateral roots. This may be due to the higher variation in lateral root diameter on nodal roots. The genotypes ‘Tx436’, ‘Koro Kollo’, and ‘Tx631’ had thin roots, while ‘CSM-63’, ‘Feterita Gishesh’, and ‘Framida’ were among the biggest root types. Interestingly, ‘Kuyuma’ had very thick PR and NRs, but very thin lateral roots, especially on NRs, while ‘Ajabsido’ behaved contrastingly. Overall, the thicker the root, the longer root hairs were measured (**Figure 4**), a trend that has also been observed in maize [69] and in rice [63].

3. Conclusion

Already, sorghum is grown in regions where maize production might fail due to adverse drought conditions. One difference between maize and sorghum lies in their root systems. The phenotypic and genotypic variation within *Sorghum bicolor*, especially for root traits such as root morphology, root hairs, and biomass, has great potential for breeding programs to mitigate climate change and to contribute to yield stability in drought prone regions with erratic precipitation. To explore the potential of the sorghum root system, we studied a subset of 30 selected genotypes, which varied in origin and breeding status, and found variation of above ground traits, but a much wider variance of root morphology, root biomass, and root hair density and length. Future experiments with resource limiting conditions will help to understand the underlying physiology of root hairs and their exudates facilitating water and nutrient acquisition, while impacting neighboring weeds and arbuscular mycorrhizal fungi.

4. Material & methods

4.1 Plant material & growth conditions

4.1.1 Plant material

For demonstration images (**Figure 1**) three different European sorghum genotypes were grown, WL08–713 and Zerberus from Germany, and SOR19 from Portugal. In the larger rhizotron screening 30 genotypes were grown, selected for variation in several physiological traits, including drought tolerance, flowering time, and origin (summarized in **Table 1**).

4.1.2 Growth in filter paper

Fungicide-coated seeds were sown either in between sheets of moist (DI water) white filter paper, placed in square petri dishes wrapped with parafilm or placed in moist brown filter paper which was rolled and placed standing upright in a 5 l container with 1 l DI water. After 10 (white) and 14 (brown) days in a greenhouse chamber with 22°C day (16 h) and 18°C night (8 h) sorghum roots were observed for root system structure, sorgoleone production and root hair formation using a stereomicroscope (MX12.5, Leica).

4.1.3 Growth in rhizotrons

Fungicide-coated seeds of 30 selected genotypes (**Table 1**) were sown in sheets of moist white filter paper, placed in a square petri dish and wrapped with parafilm. After two days a single germinated seed was placed into a rhizotron, four rhizotrons per genotype. The rhizotrons were 30x60 cm large and filled with 3 kg soil substrate (dried & sieved field soil 50:50 (v/v) mixed with dried organic soil low in nutrients “Nullerde”). Groups of six rhizotrons were grouped into a larger container, inclined to ~45° and covered to reduce light falling onto the transparent plexiglas side used for root imaging. The 30 genotypes in each replicate were randomized and grown in a greenhouse compartment at 24°C during the day (16 h, 70% humidity) and 20°C during nights (8 h, 90% humidity). After 7, 14, 18, and 21 DAS non-invasive phenotyping, and after 21 DAS invasive measurements were performed.

4.2 Non-invasive root and shoot measurements

Seedlings grown in filter paper were unwrapped gently without removing them from the paper. Roots were photographed with a digital camera and primary and their branched lateral roots imaged under a stereomicroscope (MX12.5, Leica) to evaluate sorgoleone production, root diameter, and root hair formation (length and density).

For rhizotron-grown plants, at the given time points, roots and shoots of the 30 genotypes were non-invasively measured. Shoot length was measured until the tip of the youngest elongated leaf. The root systems of rhizotron-grown plants were imaged using a photo-station equipped with a digital camera. The PaintRhizo software (FZJ) was used to mark and track primary, lateral, and nodal roots separately over time.

4.3 Invasive root and shoot measurements

4.3.1 Shoot & root dry weight

At harvest, 21 DAS and following the last non-invasive measurements, shoots were cut off, dried for seven days in a 60°C oven and then weighted to determine total shoot dry weight. After shoot removal, rhizotrons were opened and the soil was gently removed by washing using running tap water. The primary root as well as the longest crown root were gently separated from the remaining root system. From these roots several 1 cm segments (at 5, 10, 20, 30, 40 cm from the root tip) were cut and transferred to 50% ethanol (p.a.) for subsequent root morphological analyses. The remaining root system was dried for seven days in 60°C and then weighted for root dry weight determination.

4.3.2 Root morphological analyses

All root segments were imaged using a stereomicroscope (MX12.5, Leica) followed by analysis using the image J software (Fiji). Per root segment four images were taken; per image root diameter and ten root hairs were measured in length, while sorgoleone production was noted as presence or absence. Root hair density was scored following the procedure described in [63]. Distinguished were the primary root and the longest nodal root as well as their daughter roots, separated as 'thick' and 'thin' lateral roots.

4.4 Statistical analyses

The experimental data were analyzed with Excel (version 2019, Microsoft) and R (Rstudio, version 4.0.3). Genotypic variation per time point was analyzed by a one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) and the LSD (Least Significant Difference). Linear correlations over all plants was calculated as Pearson's correlation.

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Conflict of interest

The authors declare no conflict of interest.

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