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PhenoRoots: an inexpensive non-invasive phenotyping system to assess the variability of the root system architecture

Saulo Muniz Martins[1], Giovani Greigh de Brito[2], Washington da Conceição Gonçalves[2], Bruna Mendes Diniz Tripode[2], Marc Lartaud[3], João Batista Duarte[1], Camilo de Lelis Morello[2], Marc Giband[3]*

[1]Universidade Federal de Goiás/EA – Setor de Genética e Melhoramento de Plantas – Campus Samambaia, Rod. GO 462, km 0 – 74690-900 – Goiânia, GO – Brasil.
[2]Embrapa Algodão – Núcleo Cerrado, Rod. GO 462, km 12 – Zona Rural – 75375-000 – Santo Antônio de Goiás, GO – Brasil.
[3]Cirad/UMR AGAP, Av. Agropolis – 34398 – Montpellier Cedex 5 – France.
*Corresponding author <marc.giband@cirad.fr>

ABSTRACT: The root system architecture (RSA) of plants and its functioning play a fundamental role in a number of plant growth mechanisms including water and nutrient uptake. Optimization of the RSA is important for stable and increased plant productivity under adverse conditions. Despite its great importance, studying the RSA is notoriously laborious because of the difficulty of accessing the rooting system of plants. We developed a root phenotyping platform, PhenoRoots, which allows for the non-invasive study of plant RSA. The system was built using inexpensive material and was designed to provide medium throughput. Substrate or soil-filled rhizotrons are used to grow plantlets, whose roots are directly visible through a glass plate. An experiment conducted on a panel of twenty Upland cotton (*Gossypium hirsutum* L.) varieties demonstrated the usefulness of the platform in assessing RSA traits. A number of traits, destructive and non-destructive, related to the RSA were measured and statistically analyzed. The non-destructive traits based on image analysis of roots were more accurate and showed high correlation with the time-consuming destructive measurements. The platform allowed for capturing the phenotypic and genetic variability found in the panel of cotton varieties, and to define three contrasting RSA patterns. PhenoRoots provides an inexpensive alternative to the medium throughput analysis of RSA traits in plants.

Keywords: rhizotron, root traits, image analysis, medium-throughput, cotton

Introduction

Because of its role in a number of fundamental processes in plant development, a better understanding of the root system, and its development of complex interactions with the environment, and the genetic control of root system architecture (RSA) holds great promise for plant improvement. Nevertheless, due to the difficulty of securing easy and consistent access, less emphasis has been devoted to the root system than to the aerial part of plants (Herder et al., 2010), and the lack of high performance phenotyping techniques is still a bottleneck to be overcome (Joshi et al., 2017). A number of methods of studying the RSA have been developed, both destructive/invasive and non-destructive methods. The destructive methods basically consist of removing the plant from the soil or from their container in order to weigh the root, both fresh and dry (Shashidhar et al., 2012). These methods are very laborious, cause destruction of the plant and are generally not feasible for large scale experiments. The non-invasive/non-destructive methods of evaluating the RSA are characterized by the use of image processing technologies using "rootboxes" or "rhizotrons" (Neufeld et al., 1989) which facilitate studies throughout the plant's growth and development, and enable evaluation of a large number of genotypes in a much less labor-intensive way.

Advances in the field of root phenotyping have been significant in recent years as several platforms have become available (Bodner et al., 2017; Kuijken et al., 2015). Different approaches have been reported on the characterization of plant RSA, including semi-hydroponics (Chen et al., 2011), hydroponics (Courtois et al., 2013; Mathieu et al., 2015) and soil-based systems (Joshi et al., 2017; Mooney et al., 2012). The "GROWSCREEN-Rhizo" platform (Nagel et al., 2012) is a fully-automated phenotyping system that permits the simultaneous measurement of root and shoot growth for plants grown in soil-filled rhizotrons. However, most of these new technologies remain very expensive and are not suited to most public improvement programs. Bearing this in mind, we developed the "PhenoRoots" platform, a non-destructive, non-invasive, low cost rhizotron-based root phenotyping system, and in this study we present the features and validation of the RSA phenotyping platform. An experiment using 20 Upland cotton (*G. hirsutum* L.) genotypes was conducted to describe its functionalities, and we discuss its advantages over invasive/destructive methods of root assessment.

Materials and Methods

Root phenotyping platform

The root phenotyping system is constructed using commonly-found and inexpensive material, such as glass and Aluminum Composite Material (ACM) plates, and soldered iron bars. Each rhizotron consists of a 50 × 78 cm light-proof 3.5 mm thick ACM plate separated from a 50 × 80 cm glass plate (4 mm thick) by 1.2 cm thick aluminum spacers. Holes are drilled in the bottom spacer to permit drainage of excess water. The two plates are clamped together using binder clips (Figure

1A). The sandwich (outer dimension: 1.95 cm, inner dimension: 1.2 cm) is filled with substrate and clamped, glass plate side down, on a second ACM plate lined with soft rubber and mounted on a cart. The average (N = 30) weight of empty, dry substrate-filled, and saturated to field capacity rhizotrons is 6.35 ± 0.013 kg, 9 ± 0.026 kg, and 9.6 ± 0.031 kg, respectively.

The carts (2 m long and 73 cm high) are built using iron bars that are soldered together and are mounted with wheels to permit easy handling. Each cart holds 10 soft rubber-lined ACM plates on which the rhizotrons are placed, glass plate side down, ensuring that roots grow in light-proof conditions (Figure 1B), while facilitating easy visualization through the glass plate. The ACM supporting plates are positioned at a 45° angle (Figure 1B), which maximizes the proportion of roots growing against the glass plate to be visible for non-invasive analysis (Figure 2A and B). The platform, composed of 20 carts maintained in a temperature-controlled greenhouse, has the capacity to hold up to 200 rhizotrons.

Analysis of the Root System Architecture and System Validation

Plant material and growth conditions

A trial test was conducted in a temperature-controlled greenhouse maintained at a day/night temperature of 32/22 °C and an average relative humidity of 70 %. Twenty Upland cotton (*Gossipium hirsutum* L.) varieties (Table 1) were assessed. The experiment was carried out in a randomized complete block design with six replications. Other crop species, including soybean (*G. max*), bean (*P. vulgaris*), peanut (*A. hypogaea*), corn (*Z. mays*), rice (*O. sativa*), sorghum (*S. bicolor*), and pearl millet (*P. glaucum*) were also grown in the rhizotrons to demonstrate the versatility of the system.

Each rhizotron was filled with approximately 3 kg of sieved (4 mm × 4 mm apertures) potting soil enriched with 4-30-16 NPK fertilizer (375 g per 25 kg of soil) and ammonium sulfate (100 g per 25 kg). During the filling, the glass plate was gently taped with a rubber hammer to ensure even settling of the soil throughout the rhizotron. The soil-filled rhizotrons were then mounted on the carts, and fixed using plastic clamps.

Before sowing, the rhizotrons were saturated by dispensing tap water from the top of the rhizotron, and left to drain until the soil had settled to field capacity. Three seeds were sown per rhizotron the day after the soil had reached field capacity, and the rhizotrons were thinned to one plant per rhizotron by gently removing additional plants with tweezers once the plantlets had emerged.

During the first few days of seed germination, irrigation was maintained so as to keep the soil at field capacity until the plantlets' apexes had emerged from the expanded cotyledonary leaves. This was achieved by manually replenishing the amount of water that had been lost through evapotranspiration. Once the apexes had emerged, irrigation was reduced, and only half of the water lost daily was applied so as to gradually reach 80 % of the soil's field capacity (dry-down). This was usually achieved after a dry-down period of 7-8 days after sowing (DAS), at which point water was supplemented daily to maintain this level of soil moisture in the rhizotrons. To calculate the amount of water lost by evapotranspiration, five additional rhizotrons were randomly allocated in the trial and weighed every day to obtain the average daily loss of water.
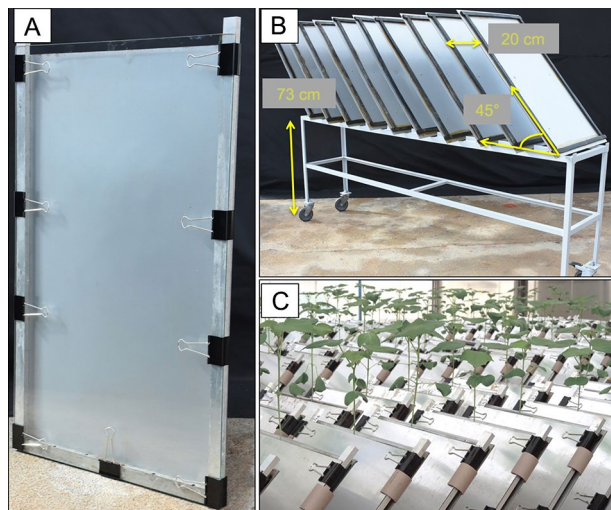

Figure 1 – Overview of the PhenoRoots phenotyping system. A) Rhizotron composed of an Aluminium Composite Material (ACM) light-proof plate (background) separated from a glass plate (foreground) by aluminum spacers. Binder clips are used to clamp the plates together. Note the holes drilled in the bottom spacers to allow excess water to drain. B) Design of the cart used to carry the rhizotrons: rhizotrons are fitted glass-side down against the ACM plates lined with soft rubber, allowing the roots to grow in the dark. C) View of the temperature-controlled greenhouse during a typical PhenoRoots experiment: one cotton plantlet is grown per rhizotron, and maintained until approximately 21 days after sowing.

Table 1 – List of the Upland cotton varieties used and their respective country of origin.

|	Genotype	Country		Genotype	Country
1	ACALA MAXXA	USA	11	FMT 701	Brazil
2	ALBAR K603	Zimbabwe	12	FMT 709	Brazil
3	BRS 269 BURITI	Brazil	13	GUAZUNCHO 2	Argentina
4	BRS 336	Brazil	14	IMACD 8276	Brazil
5	BRS 372	Brazil	15	IRMA D742	Cameroon
6	CD 408	Brazil	16	MCU 5	India
7	CD 410	Brazil	17	N`TA 90-8	Mali
8	FK 290	Burkina Faso	18	REBA P279	Paraguay
9	FM 966	Australia	19	STAM F	Togo
10	FM 993	Australia	20	STONEVILLE 474	USA
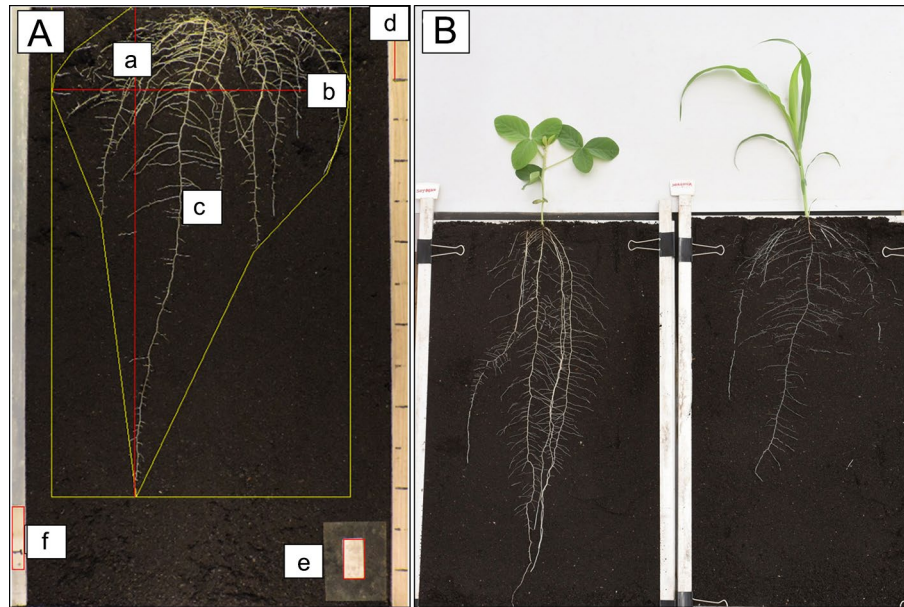
Figure 2 – Representative pictures of the root system as visualized using the PhenoRoots platform. A) Image of the root system of a cotton plant analyzed using the ImageJ toolset: the vertical axis a represents the maximum root depth (mrd); the horizontal axis b represents the maximum root width (mrw); the polygon delineated by the outermost tips of the root system (c) represents the area explored by the roots (ea); ruler (d) with a 5.0 cm increments; reference (e) of known dimensions (5 × 2.5 cm) used to convert pixels into units of length; and label (f) with plot identification. B) Representative pictures of the above-ground and root system of soybean (left) and sorghum (right) plantlets grown on the PhenoRoots platform.

Root picture acquisition, image analysis and RSA traits

The acquisition of the root images was done at 21 DAS, when the longest roots reached the bottom of the rhizotron. The rhizotrons were removed from the carts and placed, glass plate side up, on a standard photocopy stand equipped with adjustable lighting fitted in a purpose-built dark cabin. The lighting was dimmed and adjusted to a near horizontal position to avoid reflections. Pictures were taken using a digital camera with an 18-megapixel resolution (Nikon Coolpix P520) positioned at a 90° angle in relation to the plane of the rhizotrons. A ruler with 5.0 cm increments, a rectangular reference of known size (2.5 × 5.0 cm in our case) and a label indicating plot identification were placed alongside the rhizotron before the pictures were taken (Figure 2A).

Traits related to the root system architecture (RSA) assessed from the 2D RGB images are described in Table 2, and some are illustrated in Figure 2A. Data for the trait total root length (trl), average diameter of roots (adr), number of root tips (tips), projected area (pa), root surface area (rsa) and root volume (rv) were obtained using the WinRHIZO software program (WinRHIZO PRO 2013).

The maximum root depth (mrd), which measures the depth reached by the roots, maximum root width (mrw), and total area explored by the roots (ea) were obtained using a purposely-developed macro toolset running on the ImageJ software program (Abràmoff et al.,

Table 2 – Destructive and non-destructive root system traits assessed in the study, with their respective abbreviation, unit of measure and method used to obtain the data.

Trait	Abbreviation	Unit	Data obtained by
fresh root weight	frw	g	Weighing
dry root weight	drw	g	Weighing
total root length	trl	cm	WinRHIZO
average diameter of roots	adr	mm	WinRHIZO
number of root tips	tips	count	WinRHIZO
maximum root depth	mrd	cm	ImageJ
total explored area	ea	cm^2	ImageJ
maximum root width	mrw	cm	ImageJ
projected area	pa	cm^2	WinRHIZO
root surface area	rsa	cm^2	WinRHIZO
root volume	rv	cm^3	WinRHIZO
ratio mrd:mrw	mrd_mrw	-	Calculation
root density (ratio trl:ea)	trl_ea	cm cm^{-2}	Calculation

2004). A macro file ("Toolset") that contains macro tools available in ImageJ was developed to streamline image analysis and facilitate the semi-automatic measurements of root traits. The toolset includes a calibration tool ("Set Scale" tool from ImageJ) that allows for converting pixels into cm using the reference of known size (Figure 2A, letter e). Three tools are used to define the Region of Interest (ROI) for analysing the root system. An area selection tool ("Polygon" tool from ImageJ) enables the total area explored by the roots (ea) to be drawn by click-

ing on the outermost tips of the rooting system (Figure 2A letter c), and the toolset automatically calculates the maximum root depth (mrd), and the maximum root width (mrw) (Figure 2A, letters a and b respectively). The "Angle" tool from ImageJ allows for measuring the root angle, while the "Segmented Line" tool facilitates measuring the length of any given root. In addition, a counter tool ("Cell Counter" plugin available for ImageJ) enables the counting of objects such as root tips or forks. The "Results" tool activates displaying and saving the image with selections and the ROI Manager in the original image directory. All the measurements are also displayed in the "Analyses" window. The "New" tool allows for resetting the variables and opening the next image. The macro file is available upon request.

In the present study, the root angle tool, the counter tool, and root length tool were not used.

Other traits of interest for the assessment of the RSA are the product of the algebraic relationships between these variables. These include the ratio of mrd to mrw (mrd_mrw) that gives information on the distribution of the roots in the soil profile, and the ratio of trl to ea (trl_ea) which measures root density (Table 2).

At the end of the experiment, destructive measurements including the fresh root weight (frw) and the dry root weight (drw) were obtained. After the photographs had been taken, the rhizotrons were disassembled and the roots collected and washed in a fine-mesh sieve under running tap water. The washed roots were carefully blotted dry to remove excess water and weighed, then dried in an oven to determine the dry weight.

Statistical analysis

The data were submitted to descriptive and variance analyses, and the coefficient of experimental variation (CVe) was calculated as well as the selective accuracy (SA) using the following formulas: $CVe = 100 \times \sqrt{MSE} / \overline{x}$, where MSE is the Mean Squared Error from the ANOVA, and $SA = \sqrt{1 - 1/F}$, where F is the value of Snedecor's F test.

Phenotypic means of the varieties were estimated and compared between themselves using the Scott-Knott test. These means were also used to perform Pearson's correlation analysis on relationships between all the variables. Student's t-test was used to test the null hypothesis of the correlations. A heat map and dendogram were generated using the standardized phenotypic mean values of the thirteen RSA traits (Table 2) as measured in the 20 Upland cotton varieties (Table 1) using the "graphics" and "grDevices" packages. All analyses were performed in the R software environment (R Core Team, 2018).

Results and Discussion

All the plants grown in the rhizotrons showed normal development and growth of both the shoots and the roots (Figures 1C and 2A for cotton, and Figure 2B for soybean and sorghum). The positioning of the rhizotron at a 45° angle induced the roots to grow alongside the glass plate facilitating observation of the rooting system. Additional decreases in the angle (see Figure 1B) did not increase the amount of roots that could be seen (data not shown). The photographs of the root system were taken at the end of the experiment at 21 days after sowing, when the roots of the cotton plants had reached the bottom of the rhizotrons; however, since the system was devised to allow easy handling of the rhizotrons and direct access to the root system, pictures can also be taken repeatedly over the course of the experiment to provide a dynamic view of root growth and development.

Data for eleven image-based root architecture system (RSA) traits and two destructive measurments, namely fresh root weight (frw) and dry root weight (drw), were generated in the trial (Table 2). Analysis of the data showed that the PhenoRoots platform allows for the fast and non-invasive acquisition of robust data with a high degree of experimental accuracy. As shown in Table 3, the platform made it possible to capture the phenotypic variation in RSA traits present in the panel of the 20 Upland cotton varieties. With the exception of the maximum root width (mrw), the traits exhibited moderate to high magnitude variations, with a coefficient of phenotypic variation ranging from 19 % (mrd_mrw) to 60 % (rv) (Table 3).

The degree of experimental precision for the majority of the variables was high, with CVe ranging from 5 % (mrw) to 33 % (drw) (Table 4). Few traits (pa, rsa, and rv) showed CVe of higher magnitude (39 %, 39 %, and 48 %, respectively). It should be noted that these traits were computed using the WinRHIZO software program and are calculated from the "trl" and "adr" variables (Table 2). The degree of experimental precision for the non-destructive traits determined by image analysis was equal to or greater than that of the "frw" and "drw" destructive measurements (Table 4). Thus, the use of image-based methods to measure root system traits, rather than tedious time-and labor-consuming destructive methods, does not lead to lower degrees of experimental precision.

Selective accuracy (SA) has also been proposed as a statistical tool for evaluating the quality of the experiment because it simultaneously considers the magnitude of the residual variation, the number of replicates and the coefficient of genotypic variation (Resende and Duarte, 2007). These authors, consider values above 0.7 as high precision. In our experiment, the magnitude of SA ranged from 0.59 (trl_ea) to 0.87 (mrd), demonstrating that the experiment had a moderate to high degree of precision (Table 4).

Taken together, these results show that the PhenoRoots platform allows for acquiring robust data with a high degree of experimental precision. Data generated through image-based analysis are as precise or more accurate than those generated through destructive measurements, with the added benefit of they being much less labor-intensive and time-consuming.

Table 3 – Descriptive statistics[1] of the destructive and the non-destructive image-based traits related to the root system architecture of the Upland cotton varieties.

Trait[2]	Mean	Min	Max	TA	SD	CV
						%
frw	6.05	2.30	10.27	7.97	1.77	29.19
drw	0.57	0.20	1.15	0.95	0.20	35.90
trl	678.59	206.93	1404.28	1197.35	240.14	35.39
adr	1.47	0.89	2.70	1.81	0.38	25.82
tips	2736.23	1097.00	5680.00	4583.00	793.82	29.01
mrd	59.88	22.98	71.90	48.92	11.95	19.96
ea	1347.76	495.13	2369.31	1874.19	363.11	26.94
mrw	43.48	32.60	46.26	13.66	2.09	4.82
pa	98.53	29.21	296.22	267.01	45.47	46.15
rsa	309.53	91.77	930.60	838.83	142.85	46.15
rv	11.40	2.34	32.45	30.11	6.81	59.69
mrd_mrw	1.38	0.52	2.08	1.56	0.27	19.35
trl_ea	0.50	0.20	0.93	0.74	0.13	25.18

[1]Min = minimum; Max = maximum; TA = total amplitude; SD = standard deviation; CV (%) = coefficient of phenotypic variation. [2]frw = fresh root weight; drw = dry root weight; trl = total root length; adr = average diameter of roots; tips = number of root tips; mrd = maximum root depth; ea = total explored area; mrw = maximum root width; pa = projected area; rsa = root surface area; rv = root volume; mrd_mrw = ratio mrd:mrw; trl_ea = ratio trl:ea (root density).

Table 4 – Summary of the analysis of variance for the destructive and non-destructive image-based traits related to the root system architecture of the Upland cotton varieties.

Trait[1]	Mean Square				CVe	SA
	Block		Genotype			
					%	
frw	4.69	0.105ns	5.37	0.005**	24.41	0.77
drw	0.04	0.335ns	0.06	0.072ns	33.04	0.64
trl	146030.2	0.007**	98222.89	0.005**	30.53	0.75
adr	0.99	0.000**	0.16	0.042**	20.68	0.66
tips	2049635	0.001**	1091235	0.002**	24.23	0.77
mrd	253.94	0.021*	375.25	0.000**	15.83	0.87
ea	373434	0.004**	228294.8	0.004**	23.33	0.75
mrw	2.22	0.743ns	6.46	0.078ns	4.65	0.61
pa	10848.4	0.000**	2540.74	0.041*	38.52	0.66
rsa	107069	0.000**	25075.84	0.041*	38.52	0.66
rv	308.65	0.000**	47.64	0.084ns	48.36	0.60
mrd_mrw	0.13	0.011*	0.2	0.000**	14.72	0.89
trl_ea	0.02	0.194ns	0.02	0.094ns	23.77	0.59

[1]frw = fresh root weight; drw = dry root weight; trl = total root length; adr = average diameter of roots; tips = number of root tips; mrd = maximum root depth; ea = total explored area; mrw = maximum root width; pa = projected area; rsa = root surface area; rv = root volume; mrd_mrw = ratio mrd:mrw; trl_ea = ratio trl:ea (root density). ns, *, ** = not significant, significant at $p < 0.05$ and significant at $p < 0.01$ respectively. CVe = coefficient of experimental variation. SA = Selective accuracy.

The data generated by this phenotyping platform allowed for capturing the genetic variability of cotton RSA traits present in the panel of varieties that were assessed. For nine of the traits (frw, trl, adr, tips, mrd, ea, pa, rsa and mrd_mrw), a significant difference ($p < 0.05$) was observed between the genotypes (Table 4).

To exemplify the variability in root system traits found in this panel of varieties, the results of the phenotypic values (mean values over the six repetitions) are presented in Table 5. Once more, except for a few traits (drw, adr, mrw, rv, and trl_ea), differences were found between the varieties of cotton. The following varieties Reba P279, Stam F and Guazuncho 2 on the one hand, and IMACD 8276, FM 966, CD 410, FMT 701 and BRS 336 on the other, had markedly contrasting root systems. The former three varieties presented higher values than the subsequent five in eight of the thirteen traits assessed (Table 5). Thus, these three varieties, mainly Reba P279, displayed a more robust root system, through a more expanded area of contact with the soil, a more extensive area explored by the root system and a greater depth reached.

The heat map generated after a multivariate analysis using the standardized mean values of the thirteen traits defined three clusters of varieties with differing patterns of root systems (Figure 3). A first cluster, comprising the varieties Reba P279, Stam F and Guazuncho 2, displayed a rooting system with a dominant tap root, and a deeper root system exploring a greater area. A second cluster, composed of varieties CD 410, BRS 336, FMT 701, IMACD 8276 and FM 966, displayed more superficial and ramified roots. The remaining varieties consisted of a third cluster with an intermediate RSA.

Using image-based data generated on the root phenotyping platform described in this study, strong positive and significant correlation between the variables obtained by non-invasive root image analysis and variables obtained only by destructive and time-consuming methods, such as root fresh and dry weight, were observed (Table 6). For example, the destructive trait "fresh root weight" (frw) showed a series of significant high positive correlation with the image-based variables "total explored area" (ea) and "maximum root width" (mrw) values measured using the ImageJ software program as well as with the variable "total root length" (trl) as measured by the WinRHIZO software program (0.69, 0.73,

Table 5 – Adjusted means of the traits[1] related to the root system architecture of the Upland cotton varieties.

Variety	frw	drw	trl	adr	tips	mrd	ea	mrw	pa	rsa	rv	mrd_mrw	trl_ea
REBA P279	8.21 a	0.75 a	969.85 a	1.42 a	3668 a	67.50 a	1717 a	44.36 a	139.33 a	437.71 a	16.32 a	1.52 a	0.52 a
STAM F	7.81 a	0.66 a	890.88 a	1.58 a	3367 a	66.84 a	1679 a	44.12 a	141.21 a	443.62 a	17.77 a	1.51 a	0.54 a
CD 408	7.24 a	0.70 a	728.79 b	1.51 a	2763 b	56.20 b	1273 b	43.78 a	111.04 a	348.84 a	13.86 a	1.29 b	0.54 a
FM 993	7.21 a	0.69 a	740.69 b	1.24 a	3029 a	55.80 b	1359 b	43.23 a	91.61 b	287.81 b	8.94 a	1.29 b	0.53 a
GUAZUNCHO 2	6.93 a	0.60 a	941.34 a	1.64 a	3660 a	65.47 a	1561 a	43.93 a	135.93 a	427.03 a	10.27 a	1.49 a	0.62 a
FMT 701	6.61 a	0.65 a	569.32 b	1.24 a	2278 b	41.58 b	1157 b	43.65 a	71.84 b	225.70 b	7.26 a	0.96 c	0.50 a
BRS 269 BURITI	6.49 a	0.76 a	604.84 b	1.30 a	2574 b	51.69 b	1156 b	43.68 a	79.51 b	249.80 b	8.55 a	1.18 b	0.52 a
N`TA 90-8	6.18 a	0.55 a	611.44 b	1.42 a	2577 b	67.04 a	1472 a	43.85 a	86.89 b	272.98 b	9.80 a	1.53 a	0.42 a
FK 290	6.11 a	0.61 a	658.74 b	1.41 a	2791 b	63.93 a	1656 a	44.55 a	95.11 b	298.79 b	11.05 a	1.44 a	0.40 a
FMT 709	6.11 a	0.54 a	702.36 b	1.35 a	2859 b	58.46 a	1253 b	43.21 a	95.36 b	299.58 b	10.94 a	1.35 b	0.57 a
ACALA MAXXA	5.97 a	0.57 a	661.53 b	1.60 a	2733 b	63.07 a	1387 b	44.59 a	102.17 b	320.98 b	12.80 a	1.41 a	0.47 a
ALBAR K603	5.97 a	0.60 a	637.90 b	1.41 a	2576 b	58.80 a	1309 b	44.47 a	93.99 b	295.28 b	11.15 a	1.32 b	0.48 a
IRMA D742	5.95 a	0.46 a	590.45 b	1.91 a	2558 b	66.49 a	1446 a	44.41 a	103.93 b	326.50 b	15.30 a	1.50 a	0.42 a
BRS 372	5.72 a	0.60 a	811.11 a	1.49 a	3075 a	69.06 a	1347 b	43.78 a	121.69 a	382.31 a	10.27 a	1.66 a	0.57 a
BRS 336	5.31 b	0.53 a	472.04 b	1.46 a	2155 b	65.88 a	1253 b	43.57 a	64.60 b	202.96 b	7.29 a	1.51 a	0.37 a
CD 410	5.16 b	0.43 a	580.04 b	1.69 a	2269 b	67.06 a	1267 b	42.70 a	96.44 b	302.98 b	13.55 a	1.57 a	0.46 a
MCU 5	5.12 b	0.50 a	687.67 b	1.37 a	2764 b	63.40 a	1385 b	42.49 a	93.15 b	292.63 b	10.36 a	1.49 a	0.50 a
STONEVILLE 474	4.71 b	0.39 a	641.92 b	1.48 a	2616 b	54.28 b	1172 b	43.27 a	94.92 b	298.20 b	11.91 a	1.25 b	0.53 a
FM 966	3.72 b	0.32 a	503.69 b	1.46 a	1980 b	50.39 b	1038 b	40.73 a	78.99 b	248.16 b	10.76 a	1.22 b	0.49 a
IMACD 8276	3.49 b	0.37 a	539.80 b	1.25 a	2343 b	46.19 b	1060 b	41.20 a	68.89 b	216.43 b	7.17 a	1.00 c	0.47 a

[1]frw = fresh root weight; drw = dry root weight; trl = total root length; adr = average diameter of roots; tips = number of root tips; mrd = maximum root depth; ea = total explored area; mrw = maximum root width; pa = projected area; rsa = root surface area; rv = root volume; mrd_mrw = ratio mrd:mrw; trl_ea = ratio trl:ea (root density). Means followed by different letters in a column are significantly different ($p < 0.05$) according to the Scott-Knott test.

Table 6 – Phenotypic linear correlation for the traits[1] related to the root system architecture measured in the Upland cotton varieties.

	frw	drw	trl	adr	tips	mrd	ea	mrw	pa	rsa	rv	mrd_mrw
drw	0.89**											
trl	0.72**	0.56*										
adr	0.05ns	−0.24ns	0.12ns									
tips	0.73**	0.58**	0.98**	0.11ns								
mrd	0.32ns	0.13ns	0.45*	0.61**	0.48 *							
ea	0.69**	0.48*	0.72**	0.33ns	0.77**	0.73**						
mrw	0.73**	0.67**	0.43ns	0.30ns	0.50*	0.50*	0.67**					
pa	0.64**	0.40ns	0.92**	0.46*	0.88**	0.60**	0.75**	0.47*				
rsa	0.64**	0.40ns	0.92**	0.46*	0.88**	0.60**	0.75**	0.47*	1.00**			
rv	0.45*	0.14ns	0.52*	0.64**	0.46*	0.52*	0.59**	0.38ns	0.76**	0.76**		
mrd_mrw	0.27ns	0.09ns	0.42ns	0.59**	0.43ns	0.98**	0.66**	0.41ns	0.57**	0.57**	0.49*	
trl_ea	0.32ns	0.28ns	0.69**	-0.10ns	0.62**	-0.11ns	0.03ns	-0.04ns	0.57**	0.57**	0.14ns	-0.06ns

[1]frw = fresh root weight; drw = dry root weight; trl = total root length; adr = average diameter of roots; tips = number of root tips; mrd = maximum root depth; ea = total explored area; mrw = maximum root width; pa = projected area; rsa = root surface area; rv = root volume; mrd_mrw = ratio mrd:mrw; trl_ea = ratio trl:ea (root density). ns, *, ** = not significant, significant at $p < 0.05$ and significant at $p < 0.01$ respectively.

and 0.72, respectively) (Table 6). This underlines the potential of the platform to make a phenotypic assessment of the root system architecture of plants.

While developing the PhenoRoots system, we struck a compromise between the size of the rhizotron, and thus its capacity to allow plant growth over an extended period of time, and the ease of handling, and thereby the throughput of the platform. The 80 cm-high glass plates enabled cotton plants to grow for 21 days after sowing until the longest tap roots reached the bottom of the rhizotron, a stage at which cotton plants were still at a vegetative stage. Souza et al. (1983) have never-

theless shown that the selection of cotton plants at the seedling stage results in longer roots in adult plants, and the period of growth on the platform is therefore adequate for this purpose. If necessary, the rhizotrons may be resized and the platform adapted to fit larger plates to allow for the monitoring of root development over longer periods of time. However, increasing the dimensions of the rhizotrons results in increased weight and reduced ease of handling, and may affect the throughput of the system.

We successfully tested the ability of the platform to support the growth of other crop species, including
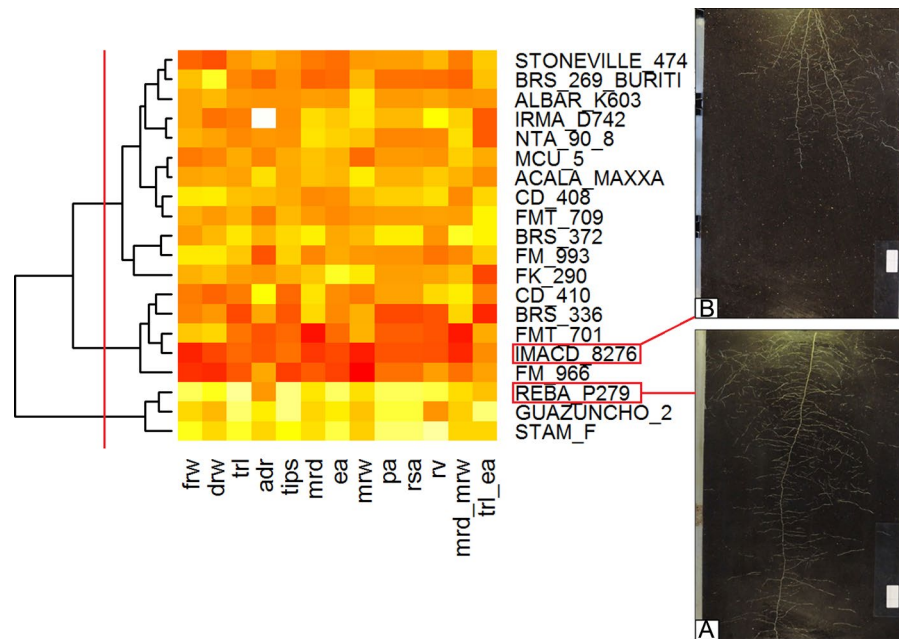
Figure 3 – Heat map and dendogram showing the cluster analysis generated using the standardized mean values of the thirteen root system architecture traits[1] measured in 20 Upland cotton varieties. Lighter colors indicate higher trait values while darker colors indicate lower values. The panels on the right show typical pictures of the root system of a variety (Reba P279) displaying a deeper system that explores a greater soil area (panel A), while panel B shows a variety (IMACD 8276) with a more superficial root system. [1]frw = fresh root weight; drw = dry root weight; trl = total root length; adr = average diameter of roots; tips = number of root tips; mrd = maximum root depth; ea = total explored area; mrw = maximum root width; pa = projected area; rsa = root surface area; rv = root volume; mrd_mrw = ratio mrd:mrw; trl_ea = ratio trl:ea (root density).

soybean (*G. max*), bean (*P. vulgaris*), peanut (*A. hypogaea*), corn (*Z. mays*), rice (*O. sativa*), sorghum (*S. bicolor*), and pearl millet (*P. glaucum*). Figure 2B shows the above-ground and the root development of soybean (left) and sorghum (right) plantlets. As for cotton, plantlet growth and development was satisfactory. In addition to the root system architecture traits that were assessed in this study on cotton, the ImageJ toolset that was developed allows for the measurement of other RSA features. In particular, in grasses such as rice (Kato et al., 2006), wheat (Manschadi et al., 2008), barley (Hargreaves et al., 2009) and maize (Singh et al., 2010), the root growth angle, which can also easily be measured using this system (not shown), has been shown to be important in a number of aspects related to plant growth and tolerance to abiotic stresses.

The "PhenoRoots" platform, a low cost root phenotyping system allows for the non-destructive analysis of RSA traits. Using Upland cotton as an example, we show that the platform facilitates capturing the phenotypic and genetic variability for the RSA traits under study present in our panel of varieties. More importantly, we showed that non-destructive image-based analyses display high correlation with destructive and time-consuming measurements.

In our particular setup, we are able to evaluate up to 200 plants in a single experiment, with an estimated hands-on time of 3-4 min per plant to document the root system. Image analysis could then be carried out over time using freely- or commercially-available image/root analysis software. This level of throughput is compatible with what is necessary in the screening and pre-breeding stages of a plant improvement program. In addition to the application described in this study, we anticipate that the PhenoRoots platform will also be useful to a study of the dynamics of root system development over time, and the effect of abiotic stresses (salt, nutrient, or water stress) on root development. As such, by facilitating the integration of phenotypic, genotypic and genomic approaches, low-cost versatile root phenotyping systems such as the PhenoRoots platform will help better apprehend the complex interactions that shape plant root systems and their adaptive responses to environmental changes.

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Authors' Contributions

Conceptualization: Brito, G.G.; Tripode, B.M.D.; Giband, M. Data acquisition: Martins, S.M.; Gonçalves, W.C.; Tripode, B.M.D.; Giband, M. Data analysis: Martins, S.M.; Duarte, J.B.; Giband, M. Design of methodology: Martins, S.M.; Duarte, J.B.; Morello, C.L.; Giband, M. Image analysis toolset development: Lartaud, M. Writing and editing: Martins, S.M.; Brito, G.G.; Duarte, J.B.; Morello, C.L.; Giband, M.

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