



RESEARCH ARTICLE

Root traits correlated with soybean yield in a subtropical region

Luciano Antônio Ebone¹, Andréia Caverzan¹, Diógenes Cecchin Silveira², José Luís Trevizan Chiomento³ & Geraldo Chavarria^{1*}

¹Laboratory of Plant Physiology, University of Passo Fundo, Passo Fundo – 99001 970, Brazil

²Department of Forage Plant and Agrometeorology, Federal University of Rio Grande do Sul, Porto Alegre – 91540-000, Brazil

³Laboratory of Olericulture, University of Passo Fundo, Passo Fundo – 99001 970, Brazil

*Email: geraldochavarria@upf.br



ARTICLE HISTORY

Received: 13 February 2023

Accepted: 01 June 2023

Available online

Version 1.0 : 03 August 2023



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (<https://creativecommons.org/licenses/by/4.0/>)

CITE THIS ARTICLE

Ebone L A, Caverzan A, Silveira D C, Chiomento J L T, Chavarria G. Root traits correlated with soybean yield in a subtropical region. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.2425>

Abstract

Root system plays a crucial role in plants' anchoring, water and nutrients acquisition and storage of carbohydrates. While it is understood that root system traits contribute to crop yield, little is known about the particular effects of root morphology over crop yield, even on major crops such as soybean (*Glycine max*). The objective of this study was to investigate if distinct soybean grain yield plants, grown at different sites, displayed differences in their root system morphology. This distinct traits in the root system should be responsible to the differences of yield, thus, showing which traits should be followed in breeding programs to develop higher yield cultivars. The soybean cultivar DM 5958 RSF IPRO was sowing in a randomized complete block design experiment. Treatments comprised three cultivation sites and five soybean grain yield classes. Plant emergence and root system morphology attributes were evaluated. Results showed that late emergence negatively influenced root development. A huge difference among sites over very thin (89%) and thin (85%) roots was found in the high yield class, and since the plants have similar yield, it appeared that the thin and very thin roots length have no impact on the yield, being influenced mostly by the environment. Forks and tips roots didn't show a pattern. In contrast, roots volume only showed a 22% reduction, demonstrating to be less influenced by the environment, resulting in a greater correlation with the grain yield. Thus identified as the main attribute to be explored when seeking to select new soybean cultivars.

Keywords

emergence; *Glycine max* (L.) Merr.; root diameter; root system morphology; WinRHIZO®

Introduction

Roots system performs numerous functions that are vital to a plant's life, including anchorage to the soil, acquisition of water and minerals, storage of carbohydrates and communication between the environment and the plant (1). Nevertheless, despite these critical functions, there are tremendous gaps in our knowledge as to how roots interact with the environment, even in major crops such as soybean [*Glycine max* (L.) Merr.], which is most widely grown legume in the world and the fourth most important crop in terms of area harvested and production worldwide (2). This is a consequence of not emphasizing root traits in breeding programs. Due to the difficulties in observing and investigating root traits like root volume, length, tips and forks in studies conducted *in situ* (1).

Even with such difficulty, identifying the particular root morphological features that distinguish high-yielding from low-yielding soybean across all growth stages is crucial for understanding how these plants achieve high yields (3). Only a small number of articles have been devoted to evaluating yield penalties related to non-uniform soybean canopies (4-5), and an even smaller number of studies have sought to understand the penalties related to non-uniform soybean roots (3). Most of these previous investigations focused on simulating stress conditions (e.g., water or nutrient deficiency), evaluating which cultivar presented a higher stress tolerance, and determining what changes occurred in the root traits (2,6-7). Being the most important result found that roots exposed to drought modify the carbon partition with the shoot, growing deeply into the soil, and increasing lateral roots' number and length (6-7) and that total length does not correlate with yield (6). These results were similar to as found in bean (*Phaseolus vulgaris* L.) where a high positive correlation between seed yield and both deep roots and deep root angles of 40° from the horizontal was found (8).

Yet the question remains: under field conditions where drought may or may not be present, what root system characteristics are present in the higher yield plants? One study attempting to answer this question found that the differences among plants with high and low yield were due to a higher root development of the higher yield plants in the late growth stages (from bloom to the full pods formation) (3), which results from changes in carbon allocation and shows a smaller root senescence (3). However, the study was based on different cultivars, and the cause of this behaviour was seen as genetic differences among the genotypes; it does not explain the differences that exist in higher and lower yield plants in the same field over the same genotype, which is the main objective of this article.

In soybean, the part of the embryonic axis from which the radicle will emerge is in close contact with the funiculus end; while this enhances interaction with the environment, it also results in a higher amount of cell deterioration in the radicle than in the plant's aerial part (9-10). Therefore, damage to deteriorated seeds growth is predominantly expressed in the roots (10). Given that deteriorated seed displays an increase in time to emerge (5),

Table 1. Minimum (Min), average (Ave), and maximum (Max) grain yield (g) per plant in the three sites (I, II, and III) for the five classes of yield.

Classes of yield	Sites								
	I			II			III		
	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max
High	31.7	36.3	70.3	28.4	32.5	47.6	29.5	36.3	66.6
Medium-high	27.0	29.3	31.7	25.0	26.8	28.3	25.8	27.1	29.3
Medium	24.3	25.8	27.0	22.6	24.1	25.0	22.3	23.9	25.8
Medium-low	21.4	23.1	24.3	19.0	21.3	22.5	18.8	20.4	22.3
Low	5.2	16.7	21.3	8.0	15.8	18.9	6.3	15.1	18.5

it raises the question: what is the impact distinct emergence times have on root growth? While distinct emergence is known to lead to dominant plants in shoot (5),

there is a lack of knowledge concerning the particular impact of late emergence in root development. We thus sought to address this gap in knowledge via the present investigation.

Specifically, the hypothesis of this exploratory research study is that with the emergence measuring, we will see that their delay negatively correlates with root growth, and that high-yield plants exhibit root system traits that are distinct from those of low-yield plants of the same genotype, showing which root traits are the most important to achieve high yield.

Materials and Methods

Plant material and experimental design

The three experimental sites were located at: (I) the municipality of Coxilha (28°07'S, 52°17'W; 721-m altitude), (II) Passo Fundo (28°13'27"S, 52°23'18"W; 696-m altitude), and (III) Passo Fundo (28°13'40"S, 52°23'32"W; 701-m altitude). The climate and soil of the three sites are the same, respectively, humid subtropical and humid dystrophic Red Latosol soil, with different chemical properties (Supplementary Table 1) and soil water content (Supplementary Figure 1).

The climatic rainfall data, relative humidity, and average temperature during the crop cycle 2018/2019 (Supplementary Figure 2) were obtained by the weather stations of the Embrapa Trigo (Wheat Brazilian Agricultural Research Corporation), located in the municipality of Coxilha and Passo Fundo.

We used the transgenic soybean cultivar DM 5958 RSF IPRO, a pure line cultivar, maturity group 5.8, of medium size and indeterminate habit of growth. The experiment was conducted in the Brazilian subtropic, in the state of Rio Grande do Sul.

To determine the five soybean grain yield classes, 200 plants from each site were collected and measured for grain yield and moisture, correcting the grain mass to 13% of moisture. Next, the collected plants were sorted from highest to lowest grain yield per plant and then divided to comprise five classes (Table 1). The experimental design was randomized complete block, with forty replications and five soybean grain yield classes in three sites, totaling fifteen treatments.

The treatments, outlined in a two-factorial scheme, consisted of three cultivation sites (I, II, and III) and five soybean grain yield classes (high, medium-high, medium,

medium-low, and low). The class yield consisted of an arbitrary division of the 200 samples collected in each field, based on grain production per plant in five yield classes.

Cultivation and analysis procedures

The experiment was performed in a no-tillage system, with oat (*Avena strigosa* Schreb.) as the previous crop. Sowing was conducted using 92% germination rate and 89% vigor seeds. Germination was determined with four samples of 100 seeds each put in wrapped wet germination paper, which were placed in a Mangelsdorf-type germination chamber and maintained at 25°C for seven days, being the number of normal seedlings read at the end of this period (11). For the vigor test, seeds were exposed at 42°C for two days, and then the germination procedure was followed (12). Seed rate was 13 seeds per linear meter with a 0.45 m row spacing, or 288.888 seeds.ha⁻¹, accompanied by a 6 kg ha⁻¹ N, 69 kg ha⁻¹ P₂O₅ and 69 kg ha⁻¹ K₂O fertilization. Seeds were inoculated with *Bradyrhizobium japonicum* (250 mL of inoculant with 7 × 10⁹ CFU/mL for 100 kg of seeds) and treated with insecticides (50 g of Fipronil for 100 kg of seeds) and fungicides (5 g of Pyraclostrobin and 45 g of thiophanate-methyl for 100 kg of seeds), according to manufacturer recommendations (BASF). Phytosanitary management was done to control pests, diseases, and weeds. The evaluated plants were collected in five different sowing lines, with 10 plants collected after every 5 m.

Plant emergence

The emergence day after sowing (EDAS) of each plant was measured daily by marking the plants with different color sticks (5). Plants were collected post-physiological maturation stage (R8), after two days of rain; the soil was dug at a depth of 30 cm, and the roots were separated from the shoot.

Root system morphology

The collected roots were washed in water to eliminate soil fragments and then digitalized by a scanner; obtained images were analyzed via WinRHIZO[®] software. The evaluated attributes were volume (V, cm³), tips (T, number), forks (F, number), and total root length (TRL, cm). Roots were grouped into different diameter classes according to their total length: very thin roots (VTR, Ø < 0.5 mm), thin roots (FR, Ø 0.5 to 2 mm), and thick roots (TR, Ø > 2.0 mm) (13).

Table 2. Anova table for the variables emergence day after sowing (EDAS), total root length (TRL), thick roots (TR), thin roots (FR), very thin roots (VTR), root volume (V), tips and forks.

Source of variation	DF	Mean square							
		EDAS	TRL	TR	FR	VTR	Volume	Tips	Forks
Replications	39	0.04	8.18	0.72	3.65	5.73	0.12	90.82	67.11
Sites (S)	2	1.32*	4090.65*	132.14*	1520.00*	3016.92*	3.77*	25970.18*	26687.09*
Yield class (YC)	4	2.26*	25.24*	16.09*	8.30*	5.55 ^{ns}	3.26*	305.24*	103.87 ^{ns}
S x YC interaction	8	0.05 ^{ns}	3.41 ^{ns}	1.05*	1.44 ^{ns}	2.55 ^{ns}	0.07 ^{ns}	134.62*	29.92 ^{ns}
Error	546	0.03	6.062	0.61	2.49	4.67	0.09	87.43	49.31
Average		7.57	124.16	23.42	38.48	62.21	3.55	830.88	534.01
CV (%)		6.99	24.09	16.54	28.43	31.36	16.19	36.49	35.31

* (significant) at 5% probability by the F test. ns (not significant) at 5% probability by the F test. Coefficient of variation (CV).

Statistical analysis

Data were subjected to the Shapiro–Wilk test for normality; data that did not show normality were transformed by the Box and Cox procedure (14). Then, analysis of variance (ANOVA) (Table 2) was applied, followed by Tukey's post-hoc test for comparisons between the means ($\alpha = 0.05$). The average of the treatments was subjected to Pearson correlation analysis; subsequently, multivariate analysis was performed by generating the Euclidean distance matrix (D²). Using cophenetic correlation analysis ($r = 0.77$), we then tested which of the hierarchical grouping methods expressed the best fit, which resulted in the choice of the unweighted pair group method with arithmetic mean (UPGMA). The number of groups was defined by the Mojena procedure (15), which proposes a calculation procedure based on the relative size of the dendrogram distances. To validate the clustering analysis, we obtained the *cophenetic* correlation coefficient, which is a measure of correlation between the elements of the original dissimilarity matrix and the matrix produced by the dendrogram, known as the cophenetic matrix (16). The relative contribution of the characters to dissimilarity between treatments was obtained by the Singh method (17).

Results

Plant emergence

We observed that soybean plants with higher yield (Table 1) emerged quickly (Table 3) with an average among the three sites of 6.86 days. On the other hand, soybean plants with lower yield (Table 1) required more time to emerge, on average 8.94 days (Table 3), which is 30.3% increase in the time required to emerge when compare with high yield plants. Due to their spatial proximity, site II and III showed a very similar emergence pattern (Table 3). No significant differences in plant stands among the sites were founded, with the sites showing plant stands between 228.960 and 232.050 plants.ha⁻¹.

Root system morphology

Differences in TRL were found among the three study sites (Table 3). Plants with higher TRLs were found in site I, those with intermediate values were found in site II, and those with the lowest values were found in site III (Table 3).

Table 3. Emergence days after sowing (EDAS), total root length (TRL) and root volume (V) of five classes of yield in three sites (I, II, and III).

Classes of yield	EDAS (days)			TRL (cm)			V (cm ³)		
	I	II	III	I	II	III	I	II	III
High	6.07 dC	7.35 cA	7.17 cA	268.1 aA	107.1 aB	46.0 Ac	4.2 aAB	3.7 aB	4.8 aA
Medium-high	6.60 cdB	7.35 cA	7.20 cA	244.4 abA	93.9 aB	37.7 aC	3.6 abB	3.6 aB	4.4 abA
Medium	7.05 bcB	7.73 bcA	7.46 bcA	235.5 abA	102.3 aB	40.1 aC	3.1 bcB	3.4 aB	4.2 abA
Medium-low	7.27 bB	8.02 bA	8.10 bA	228.7 abA	90.0 aB	38.9 aC	2.8 bcB	3.0 aB	3.8 bcA
Low	8.85 aA	8.97 aA	9.00 aA	207.2 bA	91.1 aB	33.3 aC	2.5 cB	2.2 bB	3.4 cA
CV (%)		6.9			24.0			16.1	

Note: means followed by the same uppercase letter within a row and lowercase letter within a column are not significantly different by the Tukey's test ($P = 0.05$).

The discrepancy among the sites for this variable was considerable, as soybean plants with higher grain yield presented TRLs (Table 3) that were 60% lower in site II when compared with the same class in site I. When comparing sites I and III, the differences were even greater, as plants from the same class of site III displayed an 82% reduction in TRL compared with those of site I. When compare the classes within the same site the reductions are smaller, the reductions between high and low yield were 22.7%, 14.9% and 27.6% for site I, II and III, respectively, with significantly difference only between low and high yield in site I (Table 3).

Soybean plants with high grain yield displayed larger root volumes (Table 3). Alongside with the yield per plant reduction (classes), there was a reduction in the root volumes, leading to the formation of distinct statistical groups among the classes. When comparing sites, plants cultivated in site III showed higher root volume than those in sites I and II (Table 3). Comparing the values among the sites, we can see a root volume that is 14% smaller in site I

compared to site III (Table 3) and between high and low yield the root volume reductions were 40.4%, 40.5% and 29% for site I, II and III respectively, with significantly difference among treatments in all the sites (Table 3).

Soybean plants with high grain yield cultivated in site I presented higher amounts of VTR, FR, and TR (Table 4). Moreover, regardless of the yield classes, the root system was more robust when the plants were grown in site I (Tables 3 and 4). When comparing low yield plants of site I with high yield plants of site III there was a reduction of 87.1% for VTR, 81.3% for FR and 25.3% of TR. An analysis of the relationship between FR and VTR yields the ratios 1:1.77, 1:1.81, and 1:1.82 in site I for high, medium, and low classes, respectively. For site II, these same classes presented the proportions of 1:1.26, 1:1.32, and 1:1.32, and in site III, 1:1.25, 1:1.64, and 1:1.60 (Table 4).

Plants in the medium-high yield class displayed higher tip values in site I (Table 5). However, for the forks, the high yield class showed the biggest values for all of the

Table 4. Very thin roots (VTR), thin roots (FR), and thick roots (TR) of five classes of yield in three sites (I, II, and III).

Classes of yield	VTR (cm)			FR (cm)			TR (cm)		
	I	II	III	I	II	III	I	II	III
High	145.6 Aa	46.0 aB	15.1 aC	82.2 aA	36.4 aB	12.0 aC	40.2 aA	24.6 aB	18.8 aC
Medium-high	130.8 abA	38.9 aB	11.9 aC	77.7 aA	31.4 aB	8.4 aC	35.7 abA	23.4 aB	17.3 abC
Medium	130.3 abA	46.4 aB	14.5 aC	71.6 abA	34.9 aB	8.8 aC	33.5 bcA	20.9 abB	16.7 abC
Medium-low	129.5 abA	39.1 aB	12.5 aC	69.8 abA	30.9 aB	9.8 aC	29.2 cdA	19.9 abB	16.5 abB
Low	117.5 bA	43.3 aB	12.0 aC	64.3 bA	31.4 aB	7.5 aC	25.2 dA	16.3 bB	13.8 bB
CV (%)		31.3			28.4			16.5	

Note: means followed by the same uppercase letter within a row and lowercase letter within a column are not significantly different by the Tukey's test ($P = 0.05$).

Table 5. Tips (T) and forks (F) of five classes of in three sites (I, II, and III).

Classes of yield	T (number)			F (number)		
	I	II	III	I	II	III
High	1818 abA	692 aB	352 aC	1313 aA	389 aB	154 aC
Medium-high	1979 aA	616 aB	246 aC	1182 abA	304 aB	98 aC
Medium	1453 bcA	635 aB	315 aC	1065 abA	371 aB	131 aC
Medium-low	1444 bcA	552 aB	271 aC	1129 abA	306 aB	110 aC
Low	1313 cA	549 aB	243 aC	1047 bA	322 aB	89 aC
CV (%)		36.4			35.3	

Note: means followed by the same uppercase letter within a row and lowercase letter within a column are not significantly different by the Tukey's test ($P = 0.05$).

sites (Table 5). Moreover, when comparing among the sites, the values of F and T were highest, intermediary, and lowest for sites I, II and III, respectively.

Correlation and dissimilarity

The EDAS attribute correlated negatively with all of the root characters and grain mass (Figure 1). In addition, there was a positive association between all root variables and yield per plant (Figure 1). Thus, VTR presented the lowest correlation (0.15), followed by F (0.16) and T (0.18) (Figure 1). There was a low correlation between V and TR (-0.14), and the highest correlation with grain yield was for V and FR (0.37 and 0.36, respectively); the only non-significant correlation was between V and F.

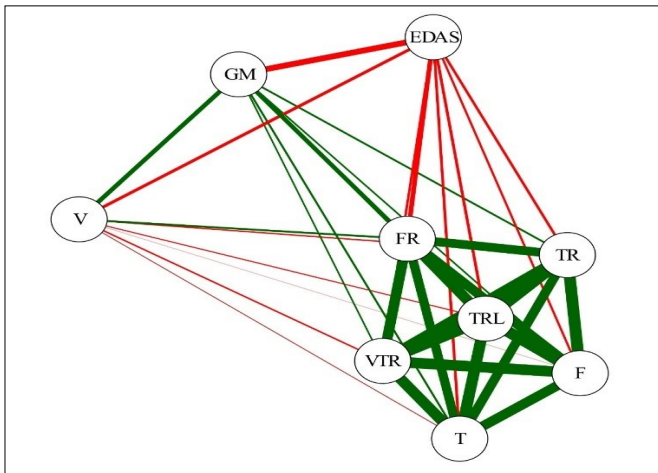


Fig. 1. Pearson's correlation coefficient for variables emergence days after sowing (EDAS), grain mass (GM), length (TRL), volume (V), tips (T), forks (F), and very thin (VTR), thin (FR), and thick roots (TR) length. Green lines represent positive correlation, and red lines negative correlations. Thick lines show significant correlations, while thin lines show no significant correlation.

The dissimilarity between the five soybean grain yield classes was illustrated in the dendrogram generated by the UPGMA method (Figure 2), whose fit to the distance matrix of the binary multicategory data, calculated by the cophenetic correlation coefficient, was 77%, indicating model adequacy. Thus, in the dendrogram obtained by the UPGMA method (Figure 2), two distinct groups were formed: one consisted only of the high yield class, and the other consisted of the other treatments. Low yield was the furthest from high yield, with $D^2 = 153.7$, while the smallest difference from high was for medium-high, with $D^2 = 23.29$.

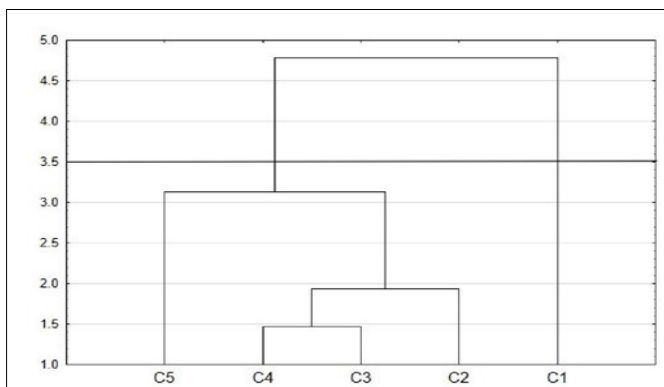


Fig. 2. Generic dissimilarity dendrogram between the five classes (high (C1), medium-high (C2), medium (C3), medium-low (C4), and low (C5) productivity) obtained by the UPGMA method, based on the average Euclidean distance matrix with length, volume, tips, forks, and very thin, thin, and thick roots length, emergence days after sowing, and grain mass. Cutoff = 3.5.

The distance from medium-high to medium was $D^2 = 3.69$, and from medium-low to low, $D^2 = 14.85$.

The traits with the highest relative contribution, according to the Singh method (16), were GM (43.1%), VTR (20.4%), TR (17.9%), and FR (15.6%), taking into account the environmental differences between the sites. When the environmental factor is excluded—that is, the differences presented due to the change of environment (soil and climate) among the sites I, II and III—the contributions according to the Singh (16) method were 90.6% for TR and 8.6% for V (Figure 3).

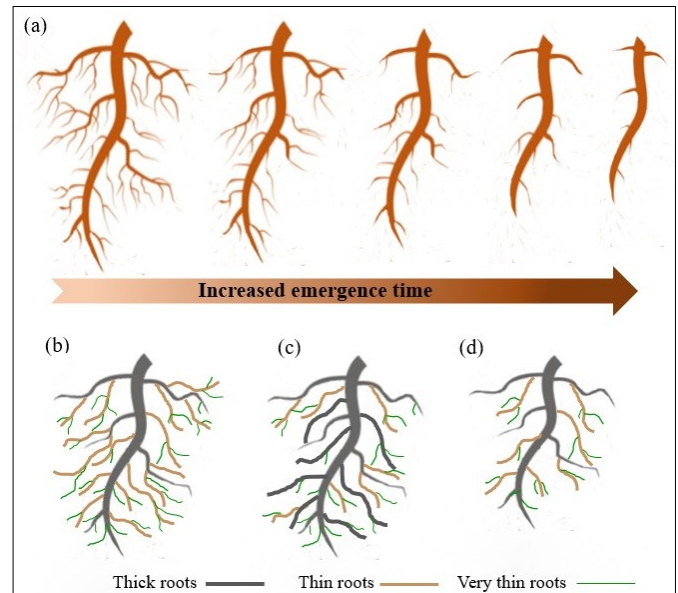


Figure 3. Influence of the root traits. (a) Increase in emergence time led to a reduction in all of the traits associated with the root system. (b) Roots with higher length of fine and very thin roots than (c), but with the same volume and thick root length, resulted in the same yield. (d) Roots with lower volume and lower length reduce the soil volume exploited by the plant, causing a reduction in plant yield.

Discussion

Our results were the first to show that the increase in plant emergence time was negatively correlated with the development of the root system and grain yield. In addition, the two main attributes responsible for plant segregation into grain yield classes were root volume and thick root's length, as demonstrated by the Singh method application (17). Discrepancies among the sites indicate that with the exception of these two attributes, the other traits studied are secondary to soybean plant yield, which is mainly affected by the environment.

The lowest EDAS values were obtained in the most productive soybean classes (Table 3), as plants that emerge earlier have higher yield (5). The mechanisms that can explain this result encompass two distinct pathways or a combination of both. It is possible that plants emerging earlier have higher yield because they have greater leaf area and in turn produce more photoassimilates, which would enhance root development leading to a high yield (5, 18).

Alternatively, the result could occur due the mechanism known as self/non-self-discrimination. This mechanism causes plants to reduce root growth in soil already

occupied by the same species roots, as this will be less efficient than growing in unoccupied soil and represent a waste of resources (19). Thus, earlier emerging plants could restrain the development of the late emerging ones by occupying the soil earlier, thereby generating a dominant and dominated situation in the roots, and leading to differences in yield and root system development (Figure 3).

EDAS was correlated with yield, showing that the special variations of soil nutrients, especially those affecting root development and the relationship with the shoot, such as N, P, and S (20), were not predominant factors in the differences presented among the roots. However, when thinking about the time required to germinate in the sites (EDAS), it is possible that nutrients content distinctions among the sites are partially responsible for that, especially potassium (K), known for having impact in many plants emergence, due to osmotic effects in germination (21). The results support the potassium impact in germination, since site I had an average EDAS (all treatments) of 7.16 days (Table 3) and a potassium concentration in the soil of 334 mg/dm³ (Supplementary table 1), site III had an average EDAS of 7.78 days and a potassium concentration in the soil of 298 mg/dm³, and site II had an average EDAS of 7.88 days and a potassium concentration in the soil of 254 mg/dm³. Nevertheless, is also necessary to take in account that site I has a soil texture with more clay and silt than the others, which improves the capacity to hold water (22), helping to achieve a quicker germination.

The root length exhibited a higher discrepancy among the sites, as its values were much more distinct from those of plant yield. Previous research showed that an increase in length did not result in an increase in yield (6). This was also found in the present work, as the TRL values were not good indicators of yield. Despite the root length not being a good indicator of yield, it impacts the plant nutrients uptake, especially in water stressed scenarios (23). However, most agricultural land present little or low water stressed conditions and the use of fertilizers made the availability of nutrients in the soil greater, so despite its importance on the uptake of nutrients, root length impact on yield is minimal over the conditions of this study.

Despite not being the focus of this work, it is necessary to address the impacts of microorganisms development over yield. The development of microorganisms occurs more where the soil conditions are better, as demonstrated by the positive correlation between phospholipid fatty acid analysis (an analysis to estimate fungi biomass and soil's bacteria biomass) and water-stable aggregates, and its negative correlation with Al, Cu and sand (24). Therefore, it is possible that the variability inside of each site is due to different soil chemistry and physical properties in the microscale, causing distinct species of microorganisms to growth in the rizosphere, which leads to yield and root development differences. Nevertheless, it is reported that high yield farms differ from the low yield ones among their microorganisms content, with high yield farms showing a greater content of the genera *Trichoderma*,

Metarhizium, *Bradyrhizobium*, *Flavobacterium* and *Duaganella*, while low yield farms showed great content of *Fusarium*, *Macrophomina* and *Septoria* (25). Thus, it is also possible that the yield and root development differences among the yield classes is being influenced by the microorganisms that grow in each plant rhizosphere. Therefore, the investigation of variability in a very small scale of the microorganisms development and content is necessary to better understand if it is a factor in distinct yield and root development inside each site.

As roots exercise different functions according to their diameter, it is imperative to subdivide them into classes (26). Generally, roots were classified into three types: very thin, thin, and thick. FR are considered more important because they are responsible for the absorption of water and nutrients, being present in plants with superior performance (27). However, our results showed that over non-restrictive conditions, plants have values of VTR and FR higher than the shoot necessity. For example, soybean plants with low grain yield cultivated in site I presented seven times more VTR and five times more FR (Table 4) than plants with double the grain yield (high class) cultivated in site III. Therefore, over normal field conditions in which humidity is generally adequate for development, plants presented a much higher root development than their necessity.

The majority of plants develop an architecture based on VTR, which allows the plant to optimize root biomass and in turn the acquisition of water and nutrients (28). Similar results were found in sites I and II (Table 4), where VTR had the highest length, followed by FR and TR. The exception was site III, where TR presented greater values in length. This could have been caused by soil compaction, which leads to hypoxia, resulting in higher ethylene production that inhibits elongation and causes a root induction, generating a root system with a higher diameter (29).

The traits of tips and forks were not useful to elucidate why roots had more or less productivity. It was expected that the number of forks indicated a more-fibrous root system (30), which could indicate a yield reduction due to the highest expenditure of photoassimilation and a less efficient resource acquisition root system. However, this hypothesis was not confirmed in our study; given the randomness of the values we obtained, we cannot make inferences about its importance.

In the present study, a great part of the data showed correlation among its elements. Mere correlation, however, is inadequate to sufficiently comprehend complex requirements such as yield and root development (31). This is despite the fact that the data indicated root volume as having the highest correlation with grain mass, signifying the importance of root volume in the plant yield within the different sites.

Nevertheless, in order to obtain a better understanding of the other components, an analysis of the relative contribution of the variables was used. This analysis showed that the variables making the greatest

contribution were, respectively, grain mass, VTR, TR, and FR. However, this contribution results from the large environmental variations among the study sites. For example, even VTR in the same class presented differences 10 times larger when comparing site I with site III, thus showing the ineffectiveness of this analysis when used in data from places with exacerbated differences among them. Therefore, separating the influence of the sites in the data becomes imperative in order to calculate the contribution of the variables. Thereby, the relative contribution of variables, excluding the differences among sites, was composed basically by TR and root volume, corroborating the analysis of the root system and reaffirming that root volume was the character most linked to yield.

Conclusion

Late emergence not only affects the development of the soybean plant aerial part, but its roots as well, with later emergence plants showing a smaller root development. It remains unknown, however, whether this phenomenon is due to the reduced aerial part growth, which could reduce the photo-assimilate availability to the root development, or to the mechanism called self/non-self-discrimination, which could generate dominated and dominant roots in the search for free soil. Our investigation found that the main attributes responsible for plant segregation into classes of grain yield were root volume and thick root length, and this should be the traits to be sought in breeding programs. Discrepancies among the sites indicate that with the exception of root volume and thick root length, the other traits studied are secondary to soybean plant yield. Very thin roots and thin roots had huge differences among sites, but resulted in plants with similar yield, showing their secondary role in the yield. The study also found that very thin roots generally had higher length values. However, new researches need to be developed to see if root volume is the major trait for others cultivars, soils and managements. Also new research is necessary to improve the actual acknowledge of the impacts of emergence in roots, if it is a question about photo-assimilates availability or a dominant/dominated situation.

Acknowledgements

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Authors contributions

Conceptualization, L.A.E.; Data curation, L.A.E., A.C.; Formal analysis, D.C.S.; Funding acquisition, G.C.; Investigation, L.A.E. and A.C.; Project administration, A.C. and G.C.; Supervision, L.A.E., G.C.; Visualization, L.A.E.; Writing—original draft, L.A.E.; Writing—review & editing, A.C., J.L.T.C., and G.C. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Supplementary data

Supplementary Table 1. Soil analysis of the three sites (I, II, and III) of the experiment.

Supplementary Table 2. Experimental results.

Supplementary Fig. 1. Soil water content. Volume moisture (m³/m³) at field capacity (Vm FC) and permanent wilting point (Vm PWP) in different soil depths (0.00–0.20 m) at three sites (I, II, and III).

Supplementary Fig. 2. Climate data. Rainfall (mm), radiation (calories/cm²-day⁻¹), and average temperature of air (°C) during the cycle of the crop (from November 2018 to April 2019.) in (A) site I, (B) site II and site III.

References

- Ryan PR, Delhaize E, Watt M, Richardson AE. Plant roots: understanding structure and function in an ocean of complexity. *Ann Bot.* 2016;118(4):555-59. <https://doi.org/10.1093/aob/mcw192>
- Fried HG, Narayanan S, Fallen B. Evaluation of soybean [*Glycine max* (L.) Merr.] genotypes for yield, water use efficiency and root traits. *PLoS One.* 2019;14(2):e0212700. <https://doi.org/10.1371/journal.pone.0212700>
- Jin J, Wang G, Liu X, Mi L, Li Y, Xu X, Herbert SJ. Genetic improvement of yield shapes the temporal and spatial root morphology of soybean (*Glycine max*) grown in north-east China. *N Z J Crop Hortic Sci.* 2010;38(3):177-88. <https://doi.org/10.1080/01140671.2010.495375>
- Masino A, Rugeroni P, Borrás L, Rotundo JL. Spatial and temporal plant-to-plant variability effects on soybean yield. *Eur J Agron.* 2018;98:14-24. <https://doi.org/10.1016/j.eja.2018.02.006>
- Ebone LA, Caverzan A, Tagliari A, Chiomento JLT, Silveira DC, Chavarria G. Soybean seed vigor: Uniformity and growth as key factors to improve yield. *Agronomy.* 2020;10(4):545. <https://doi.org/10.3390/agronomy10040545>
- He J, Jin Y, Du YL, Wang T, Turner NC, Yang RP, Li FM. Genotypic variation in yield, yield components, root morphology and architecture, in soybean in relation to water and phosphorus supply. *Front Plant Sci.* 2017;8:1499. <https://doi.org/10.3389/fpls.2017.01499>
- Kunert KJ, Vorster BJ, Fenta BA, Kibido T, Dionisio G, Foyer CH. Drought stress responses in soybean roots and nodules. *Front Plant Sci.* 2016;7:1015. <https://doi.org/10.3389/fpls.2016.01015>
- Binaissa ULC, Kimurto PK, Ojwang PPO. Stability of common bean (*Phaseolus vulgaris* L.) genotypes for root system architecture and seed yield in multi-environments. *Field Crops Res.* 2022;293:108863. <https://doi.org/10.1016/j.fcr.2023.108863>
- Hartmann-Filho CP, Goneli ALD, Masetto TE, Martins EAS, Oba GC. The effect of drying temperatures and storage of seeds on the growth of soybean seedlings. *J Seed Sci.* 2016;38:287-95. <https://doi.org/10.1590/2317-1545v38n4161866>
- Ebone LA, Caverzan A, Chavarria G. Physiologic alterations in orthodox seeds due to deterioration processes. *Plant Physiol Biochem.* 2019;145:34-42. <https://doi.org/10.1016/j.plaphy.2019.10.028>

11. Brasil-Ministério da Agricultura, Pecuária e Abastecimento. Regras Para Análise de Sementes, 1st ed. Brasília: Ministério da Agricultura, Pecuária e Abastecimento, Secretária de Defesa Agropecuária. 2009;147-220.
12. Krzyzanowski FC, Vieira RD, França-Neto JB. Vigor de Sementes: Conceitos e Testes, 1st ed. Londrina: Associação Brasileira de Tecnologia de Sementes-ABRATES;1999.
13. Chiomento JLT, De Nardi FS, Filippi D, Trentin TS, Dornelles AG, Fornari M, Nienow AA, Calvete EO. Morpho-horticultural performance of strawberry cultivated on substrate with arbuscular mycorrhizal fungi and biochar. *Sci Hortic.* 2021;282:e-110053. <https://doi.org/10.1016/j.scienta.2021.110053>
14. Box GEP, Cox DR. An analysis of transformations. *J R Stat Soc.* 1964;39:211-52. <https://doi.org/10.1111/j.2517-6161.1964.tb00553.x>
15. Mojena R. Hierárquical grouping method and stopping rules: an evaluation. *Comput J.* 1977;20:359-63. <https://doi.org/10.1093/comjnl/20.4.359>
16. Sokal RR, Rohlf FJ. The comparison of dendrograms by objective methods. *Taxon.* 1962;11:33-40. <https://doi.org/10.2307/1217208>
17. Singh D. The relative importance of characters affecting genetic divergence. *Indian J Genet Plant Breed.* 1981;41:237-45.
18. Raza MA, Gul H, Hasnain A *et al.* Leaf area regulates the growth rates and seed yield of soybean (*Glycine max* L. Merr.) in intercropping system. *Int J Plant Prod.* 2022;16:639-52. <https://doi.org/10.1007/s42106-022-00201-8>
19. Falik O, de Kroon H, Novoplansky A. Physiologically-mediated self/non-self root discrimination in *Trifolium repens* has mixed effects on plant performance. *Plant Signal Behav.* 2006;1(3):116-21. <https://doi.org/10.4161/psb.1.3.2639>
20. Ericsson T. Growth and shoot: root ratio of seedlings in relation to nutrient availability. *Plant Soil.* 1995;168:205-14. <https://doi.org/10.1007/BF00029330>
21. Follmer CM, Hummes AP, Lângaro NC *et al.* Nutrient availability and pH level affect germination traits and seedling development of *Conyza canadensis*. *Sci Rep.* 2021;11:15607. <https://doi.org/10.1038/s41598-021-95164-7>
22. Sun C, Zhao W, Liu H, Zhang Y, Zhou H. Effects of textural layering on water regimes in sandy soils in a desert-oasis ecotone, Northwestern China. *Front Earth Sci.* 2021;9:627500. <https://doi.org/10.3389/feart.2021.627500>
23. He J, Jin Y, Du YL, Wang T, Turner NC, Yang RP, Siddique KHM, Li FM. Genotypic variation in yield, yield components, root morphology and architecture, in soybean in relation to water and phosphorus supply. *Front Plant Sci.* 2017;8:1499. <https://doi.org/10.3389/fpls.2017.01499>
24. Mann C, Lynch D, Fillmore S, Mills A. Relationships between field management, soil health and microbial community composition. *Appl Soil Ecol.* 2019;144:12-21. doi: <https://doi.org/10.1016/j.apsoil.2019.06.012>
25. Bandara AY, Weerasooriya DK, Trexler RV, Bell TH, Esker PD. Soybean roots and soil from high- and low-yielding field sites have different microbiome composition. *Front Microbiol.* 2021;12:675352. <https://doi.org/10.3389/fmicb.2021.675352>
26. Liu Y, Wang G, Yu K, Li P, Xiao L, Liu G. A new method to optimize root order classification based on the diameter interval of fine root. *Sci Rep.* 2018;8:2960. <https://doi.org/10.1038/s41598-018-21248-6>
27. Souza TC, Magalhães PC, Castro EM, Duarte VP, Lavinsky AO. Corn root morphoanatomy at different development stages and yield under water stress. *Pesquisa Agropecuária Brasileira.* 2016;51(4):330-39. <https://dx.doi.org/10.1590/S0100-204X2016000400005>
28. Wu Q, Pagès L, Wu J. Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Ann Bot.* 2016;117(3):379-90. <https://doi.org/10.1093/aob/mcv185>
29. Sarto MVM, Bassegio D, Rosolem CA, Sarto JRW. Safflower root and shoot growth affected by soil compaction. *Bragantia.* 2018;77(2):348-55. <https://doi.org/10.1590/1678-4499.2017191>
30. Prince SJ, Murphy M, Mutava RN, Zhang Z, Nguyen N, Kim YH, Pathan SM, Shannon GJ, Valliyodan B, Nguyen HT. Evaluation of high yielding soybean germplasm under water limitation. *J Integr Plant Biol.* 2016;58:475-91. <https://doi.org/10.1111/jipb.12378>
31. Kanbar A, Toorchi M, Shashidhar H. Relationship between root and yield morphological characters in rainfed low land rice (*Oryza sativa* L.). *Cereal Res Commun.* 2009;37(2):261-68. <https://doi.org/10.1556/crc.37.2009.2.14>