GMR The Original

Genotypic Diversity in Root Architectural Traits and Adaptation of Wheat to Water-Limited Environmental Conditions

V. Carpentieri-Pipolo[1]*; E. Caierão[1]; R. L. Castro [1]; P. L. Scheeren [1]; V. Fronza [1], G. A. Dalmago[1], J. S. Sobrinho[1], J. P. Silva Junior[1], F. M. Santana[2], T. A. M. Kiihl[1], W. Q. Ribeiro Junior[3]

1 Embrapa Trigo, Rodovia BR 285, Km 294, 99050-970, Passo Fundo, RS.
2 Embrapa Clima temperado, BR-392, km 78, 96010-97, Monte Bonito, RS.
3 Embrapa Cerrados BR-020, km 18, 73310-970, Brasília - DF,

Corresponding Author: Valéria Carpentieri-Pipolo
E-mail: valeria.carpentieri-pipolo@embrapa.br

ABSTRACT

In wheat, the seminal root angle is representative of the root architecture of the adult plant, making it a useful tool because it is a trait that can be easily phenotyped in the early stages of plant development. A narrow seminal root angle can increase access to residual moisture at depth, particularly under terminal drought conditions. The objective of this work is to evaluate the genetic divergence of root architecture traits and to present a high-throughput root architecture phenotyping method that can be easily implemented in breeding programs. Wheat seedlings were evaluated for root architecture in a system of hanging file folders covered with germination paper. For each plant, measurements were taken of the angle between the first two seminal roots, length, and number of seminal roots. Based on the drought-tolerant control BRS 404, which presented an angle of the first pair of seminal roots of 54.73°, roots with an angle less than 60° were classified as narrow angles and were selected as standard selection criteria. The distances between accessions ranged from 0.165 to 28.64, allowing the formation of eight diversity groups. The traits that contributed most to the divergence were plant score (69.48%) and the angle of the first pair of seminal roots (24.75%). The transgenic lines Cadenza HB4® x BRS 264 and Cadenza HB4® x BRS 404, cultivars and lines BRS 404 and BR_18 UB2.323.916, UB2.323.907, UB2.323.917, UB2.323.919, with a narrow angle between the first pair of seminal roots and a plant score above 5, were selected as potential for planting under water-restricted environmental conditions. The method allowed high differentiation between genotypes and is therefore suitable for large-scale screening with high throughput of root architecture traits.

Keywords: *Triticum aestivum, plant breeding, root angle, root number, adaptation.*

INTRODUCTION

Drought is the main and most important limiting factor for wheat (*Triticum aestivum* L.) production worldwide. Water deficits during critical periods of crop development, such as grain filling, can significantly impact yield stability and productivity in rainfed agricultural systems. Climate change is expected to affect crop geography and particularly alter the Brazilian agricultural landscape, where most areas are cultivated under rainfed conditions. The area planted with wheat in Brazil in 2024 was 3.0 million hectares, with a production of 7.9 million tons of grain, representing 66% of domestic consumption, which is 12 million tons (CONAB, 2025).

The demand for imported wheat for domestic supply is around 4 million tons, impacting the trade balance (CONAB, 2025). Traditional wheat breeding relies heavily on selection for yield. However, the rate of genetic progress has declined in recent years. Yield is a quantitative trait, characterized by low heritability and high genotype-by-environment (G × E) interactions, especially in drought-affected environments.

Studies related to drought tolerance will be increasingly strategic for the country, as this is one of the abiotic stresses with the greatest impact on crops and is also the main factor expected to limit global food production in the coming years. In this scenario, in addition to crop management alternatives, the introduction of morphological traits for adaptation to water stress into breeding programs becomes imperative as an innovative solution.

In wheat, the seminal root angle is representative of the root architecture of the adult plant, making it a useful tool because it is a trait that can be easily phenotyped in the early stages of plant development. For example, a narrow seminal root angle is associated with high proportions of deep-rooting in later stages of mature wheat (de Dorlodot et al., 2007, El Hassouni et al., 2018). A narrow seminal root angle can increase access to residual moisture at depth, particularly in terminal drought conditions (Manschadi et al., 2008, Reynolds et al., 2007), and can prolong grain filling and increase yield. Conversely, an wide seminal root angle is associated with a shallow root system that can benefit the superficial exploration of soil layers and the absorption of seasonal rainfall. Therefore, identifying the optimal root angle architecture in each target environment is critical for guiding breeding efforts (El Hassouni et al., 2018).

Despite the availability of high-throughput phenotyping methodologies capable of evaluating a large number of genotypes in a short period of time, implementation in most breeding programs for the selection of genotypes with drought-stress tolerance traits still presents bottlenecks.

Several methods for phenotyping root traits have been developed in the field and laboratory, such as soil sampling (Wasson et al., 2012); thermography (Lopes et al., 2010); X-ray tomography (Mairhofer et al., 2013); and rhizotrons (Lobet & Draye, 2013). Many of these methods are laborious, expensive, and do not allow for the characterization of a large number of accessions (high-throughput phenotyping) aimed at identifying drought-stress tolerance genes to be incorporated into breeding programs.

The objective of the study is to evaluate the genetic divergence of root architecture traits and to present a high-throughput, low-cost, and easy-to-implement root architecture phenotyping method in breeding programs.

MATERIAL AND METHODS

Genetic Material

Thirty wheat genotypes from breeding programs of different origins and adaptation regions were used as follows. Ten wheat lines and cultivars recommended for planting in adaptation region 4 (hot and dry region) (Cunha et al., 2006) under water-restricted conditions: BRS 404, BR18, MGS 1 Aliança, BH1146, IAC 5 Maringá, CD 104, MGS 3 Brilhante, Embrapa 2, Cadenza HB4® x BRS 264, Cadenza HB4® x BRS 404. The lines Cadenza HB4® x BRS 264 and Cadenza HB4® x BRS 404 lines are derived from transgenic wheat that contains the sunflower *Hahb4* drought tolerance gene.

Five lines from the breeding program for adaptation region 4 (hot and dry region): UB2.323.917, UB2.323.918, UB2.323.916, UB2.323.919, UB2.323.907.

Five cultivars recommended for planting in adaptation region 4 in an irrigated system: CPAC0632, BRS 394, Embrapa 22, BRS 264, BRS 254. Five lines from the breeding program for adaptation region 3 (humid region with an average temperature of the coldest month equal to 12°C): PF200171, PF190387, PF190411, PF190407, PF210196 and Five synthetic lines introduced from CIMMYT (International Maize and Wheat Improvement Center): CIGM872771, CIGM93.388, CASW96Y575S, CASW96Y579S, CASW96Y526S.

The cultivar BRS 404 and the cultivar BRS 394, tolerant and susceptible, respectively, to cultivation under water restriction, were used as controls.

Seedling cultivation in a hanging file folder system.

Wheat seedlings were evaluated for root architecture in a hanging file folder system (Hund et al., 2009). The folders were covered on both sides with sheets of germination paper. The seeds were disinfected with 0.5% sodium hypochlorite for 5 minutes and glued to the upper end of the folders, on top of the germination paper sheets, ensuring that the seed embryo was placed face down to allow the roots to grow along the folders. The folders were suspended vertically in plastic boxes containing water. Water was added to the boxes in sufficient quantity to moisten the lower end of the folders. The experiment was conducted in a greenhouse at 23°C in a randomized complete block design with six replicates.

After 10 days, the folders were opened, and images were taken using a camera. For each plant, measurements were taken of root architecture characteristics, the angle between the first two seminal roots, and the length and number of seminal roots. For each plant, the angle between the first two seminal roots was measured approximately 3 cm from the seed using ImageJ software (NIH National Institutes of Health, USA) (Figure 1).

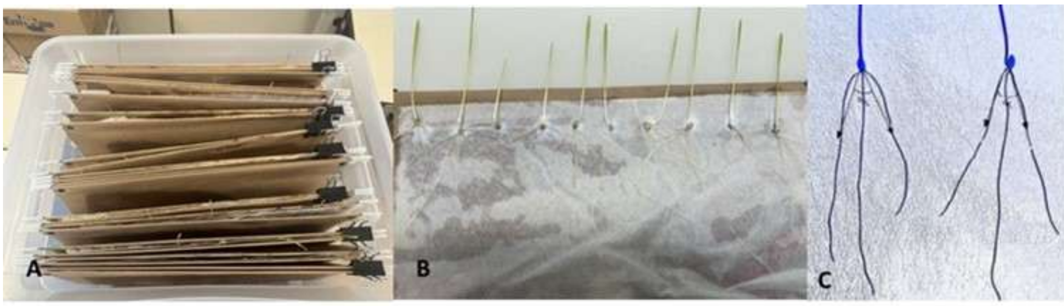

Figure 1. (A) Evaluation of root architecture in wheat seedlings using the suspended file folder method. Folders suspended vertically in plastic boxes containing water. (B) Wheat seedlings, 10 days after sowing. (C) For each plant, the angle between the first two seminal roots was measured approximately 3 cm from the seed. 2025 and Passo Fundo, RS

The genotypes were evaluated in the field under cerrado conditions in Uberaba (Minas Gerais) using a plant score (P) scale ranging from 0 to 10, with 0 being the least adapted and 10 being the most adapted.

For statistical analysis of the data obtained in the experiment, analysis of variance and Pearson's correlation coefficient were performed (Cruz et al. 2014). To compare means, the Scott-Knott test was used. To estimate the genetic dissimilarity between pairs of accessions, a Mahalanobis matrix (D2) was calculated. Based on this matrix, cluster analysis and the Tocher optimization method (Cruz et al., 2014) were obtained using the GENES program (Cruz, 2001).

RESULTS AND DISCUSSION

Based on the information obtained through analysis of variance, a significant difference was observed between the contrasting genotypes for the angle between the first two seminal roots, root length, and number of roots. The overall mean seminal root angle was 73.03°, with a range from 40.00° to 105.07°. The cultivar BRS 404, tolerant to water deficit, were used as controls recommended for adaptation region 4 (Cunha et al., 2006) under water restriction conditions, and the BRS 394, susceptible to water déficit, used as control, recommended for adaptation region 4 under irrigated conditions, presented angles between the first pair of seminal roots of 54.73° and 84.00°, respectively.

Based on the controls, the selection criteria were standardized: roots with an angle less than 60º were classified as narrow angle, between 61º and 83º were classified as intermediate angle and above 84º were classified as wide angle (Table 1, Figure 1).

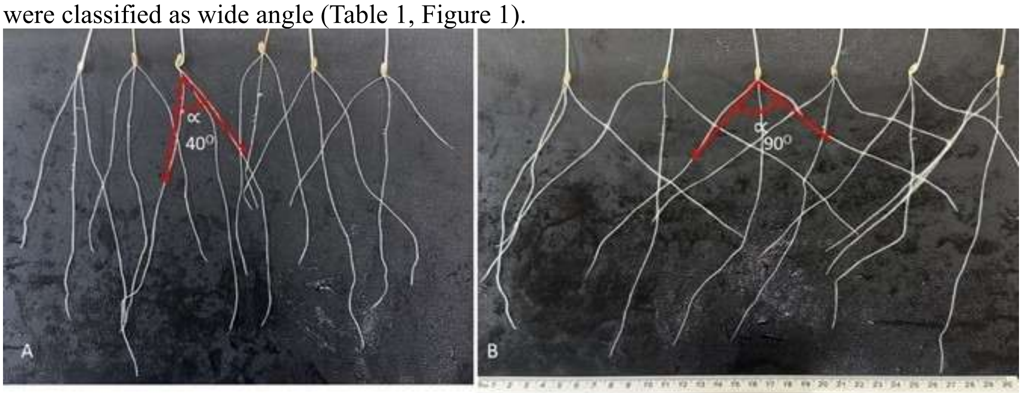

Figure 1. (A) Wheat cultivar (*Triticum aestivum* L.) with a narrow angle between the first two seminal roots. (B) Wheat cultivar with an wide angle between the first two seminal roots. 2025, Passo Fundo.

Table 1. Average angle between the first pair of seminal roots, length, number of roots, plant score, angle classification and adaptation region of wheat genotypes. 2025, Passo Fundo, RS.

Genotypes	Angle between the first pair of seminal roots (°)		Root length (cm)		Root number		P		Angle classification	Region of adaptation (1)
BR 18	59,47	d	17,32	a	3,67	a	6,58	b	narrow	
MGS1Aliança	64,67	c	15,70	a	3,33	b	5,65	d	intermediate	
BH 1146	68,95	c	17,18	a	3,33	b	5,58	e	intermediate	Region 4
IAC 5 Maringá	66,74	c	16,40	a	3,50	a	5,53	e	intermediate	dry
MGS3 Brilhante	64,61	c	16,00	a	3,17	b	5,50	d	Intermediate	(Cultivars)
Embrapa 21	64,14	c	17,43	a	3,33	b	5,65	d	Intermediate	
BRS 404	54,73	d	16,13	a	4,00	a	8,01	a	narrow	
CD104	57,73	d	17,76	a	3,66	a	5,83	c	narrow	
CadenzaHB4 x BRS264	57,26	d	14,02	b	3,50	a	7,76	a	narrow	
CadenzaHB4 x BRS404	58,37	d	18,78	a	4,00	a	7,81	a	narrow	
UB2.323.916	54,54	d	17,09	a	3,00	b	5,88	c	narrow	Region 4
UB2.323.917	42,66	e	18,68	a	3,00	b	5,80	c	narrow	dry
UB2.323.918	68,39	c	11,07	b	3,00	b	5,81	c	Intermediate	(Lines)
UB2.323.919	40,00	e	15,76	a	3,00	b	5,81	c	narrow	
UB2.323.907	57,73	d	15,63	a	3,00	b	5,76	c	narrow	
CPAC0632	103,61	a	16,23	a	3,33	b	4,75	e	wide	
Embrapa 22	105,07	a	17,43	a	3,00	b	5,01	c	Wide	
BRS 264	101,30	a	17,38	a	3,83	a	4,80	e	Wide	Region 4

BRS 254	84,40	b	14,39	b	2,83	b	4,90	e	Wide	Irrigated
BRS394	84,00	b	15,78	a	3,50	a	4,85	e	Wide	(Cultivars)
PF200171	87,63	b	13,08	b	3,50	a	4,90	e	Wide	
PF190387	84,83	b	19,47	a	3,83	a	4,83	e	Wide	
PF190411	90,54	b	17,00	a	3,67	a	4,61	e	wide	Region 3
PF190407	93,99	a	16,27	a	3,83	a	4,73	e	wide	humid
PF210196	97,83	a	16,67	a	3,50	a	4,51	e	wide	(Lines)
CIGM872771	72,01	c	17,32	a	4,00	a	5,76	c	Intermediate	
CIGM93388	65.62	c	17,88	a	4,00	a	5,83	c	Intermediate	
						a	5,76	c		CYMMIT
CASW96Y575S	66,81	c	16,48	a	4,00				Intermediate	lines
CASW96Y579S	74,96	c	17,90	a	3,17	b	5,86	c	Intermediate	
CASW96Y526S	72,64	c	15,69	a	3,17	b	5,80	c	Intermediate	

Means followed by the same letter do not differ statistically from each other by the Scott-Knott test at a 5% probability level.

(1) Evaluation at the seedling stage using the suspended file folder method

P = Plant Score. Evaluation carried out at maturity, in the Cerrado region, in Uberaba (MG), scale from 1 to 10, where 1 is less adapted, 9 is adapted.

(2) Wheat adaptation regions (Cunha et al., 2006).

Regarding root growth angle, the cultivars BRS 264, Embrapa 22, and the lines CPAC0632, PF210196, and PF190407 were similar to each other according to the Scott-Knott mean comparison test at 5% probability and presented the highest values (ranging from 93.99 to 105.07) for the angle between the first pair of seminal roots, being classified as wide angle.

The cultivar BRS 254, recommended for irrigated sowing, and the lines PF200171, PF190387, and PF190411 recommended for region 3 (humid) according to the Scott-Knott mean comparison test at 5% probability, were similar to each other and presented an wide angle between the first pair of seminal roots equal to the control BRS 394 (84.00°), sensitive to water deficit.

The synthetic lines introduced from CIMMYT (International Maize and Wheat Improvement Center) CIGM872771, CIGM93388, CASW96Y575S, CASW96Y579S, CASW96Y526S, cultivars MGS1 Aliança, BH 1146, IAC 5 Maringá, Embrapa 21, MGS3 Brilhante and line UB2.323.918 all recomended for sowing under rainfed in region 4 (hot and dry), according to the Scott-Knott mean comparison test at 5% probability, were similar to each other and presented an angle between the first pair of seminal roots ranging from 64.14 to 74.96, being classified as intermediate. The wheat cultivars and lines recommended for sowign under rainfed condition in adaptation region 4 (Cunha et al, 2006) under water restriction conditions BR18, CD 104, Cadenza HB4® x BRS264, Cadenza HB4® x BRS 404, the lines of the breeding program for adaptation region 4 (hot and dry region) UB2.323.916, UB2.323.907 according to the Scott Knott comparative test of means at 5% probability, were similar to each other and presented an narrow angle between the first pair of seminal roots equal to the control BRS 404 (54.73º) tolerant to water deficit (Table 1).

The Cadenza HB4® x BRS264 and Cadenza HB4® x BRS 404 lines presented the highest plant field scores for adaptation to the cerrado region, 7.76 and 7.81, respectively, being, according to the Scott-Knott mean comparison test at 5% probability, similar to the water deficit-resistant control BRS 404 (8.01) (Table 1). The BR18 cultivar, tolerant to water deficit, presented a plant field score of 6.58.

The lines adapted to region 4: UB2.323.916, UB2.323.917, UB2.323.918, UB2.323.919 and UB2.323.907, the Embrapa 22 cultivar, and the introduced lines of CIMMYT CIGM872771, CIGM93388, CASW96Y575S, CASW96Y579S, and CASW96Y526S were similar to each other according to the Scott-Knott mean comparison test at 5% probability and presented plant field scores ranging from 5.01 to 5.88.

Embrapa 21 and MGS3 Brilhante were similar to each other according to the Scott-Knott mean comparison test at 5% probability, presenting plant field scores of 5.65 and 5.50, respectively. The cultivars recommended for irrigated sowing, CPAC0632, BRS264 and BRS254, and the lines PF200171, PF190387, PF190411, PF190407 and PF210196 adapted to the humid region were similar to each other according to the Scott-Knott mean comparison test at 5% probability with plant field scores ranging from 4.51 to 4.90 (Table 1).

Most of the narrow angles were found in cultivars and lines recommended for sowing under rainfed conditions at Region 4, which are part of a collection that represents the genetic diversity of regional wheat varieties from the Cerrado region, which is characterized but a hot and dry environment. The overall average for root length was 15.34 cm with a range of 11.07 to 19.47 cm. The overall average for the number of seminal roots was 3.45 with a range of 2.83 to 4.00 (Table 1). Among the evaluated genotypes, a small difference was observed in the number of roots characteristic. This small difference is consistent due to the low variation in the maximum number of seminal roots that can occur in wheat, which is 5 or 6 roots (Sanguineti et al., 2007).

Pearson's correlation analysis was significant at the 5% probability level between the angle between the first two seminal roots and plant score, and between length and number of seminal roots. Correlations between the other characters were not significant. In this work, the greenhouse study results were consistent with the field findings. Work performed on seedlings showed that the first pair of seminal roots was correlated with the adult plant field score. The angle at which roots emerge from the seed and penetrate the soil determines root system architecture (Wasson et al., 2012)

The present study indicates that root architecture variability exists between the genotypes across the groups, as well as within groups. The distances between the accessions ranged from 0.165 to 28.64, allowing the formation of eight diversity groups (Table 2). The characters that contributed most to the divergence between genotypes were plant score (69.48%), angle of the first pair of seminal roots (24.7%), and root length (4.06%).

Table 2. Grouping of 30 wheat genotypes based on the angle of the first pair of seminal roots, length, root number and plant score by the Tocher method, using Mahalanobis generalized distance as a measure of genetic distance. 2025. Passo Fundo, RS.

Group	
<1>	CD104 MGS1_Aliança BH_1146 IAC _5_Maringá MGS_3_Brilhante Embrapa_21 UB2.323.916 UB2.323.907 CIGM872771 CIGM93388 CASW96Y575S CASW96Y579S CASW96Y526S BH_1146 IAC _5_Maringá
<2>	PF_190387 PF_190411 PF_190407 PF_210196 CPAC_0632 BRS_264 BRS_394
<3>	BR_18 UB2.323.919 UB2.323.917
<4>	BRS_404 Cadenza_ HB4® xBRS_264 Cadenza_ HB4® xBRS_404
<5>	UB2.323.918
<6>	Embrapa_22
<7>	PF_200171
<8>	BRS_254

Group 1 included 16 wheat genotypes with the angle of the first pair of seminal roots ranging from 54.54º to 74.96º, classified as intermediate and narrow angle of the first pair of seminal roots. Notable genotypes grouped in Group 1 include cultivars recommended for planting in adaptation region 4 (Cunha et al., 2006) under water-restricted conditions, lines from the breeding program for adaptation region 4 (hot and dry region), and synthetic lines introduced from CIMMYT (International Maize and Wheat Improvement Center).

Wheat genotypes with the narrowest distribution of lateral roots can access more moisture at depth in the soil profile (Manschadi et al., 2008). A more vertical angle of the seminal roots and a greater number of seminal roots are associated with a more compact root system with a greater number of roots at depth

(Bengough et al., 2004; Manschadi et al., 2008). Narrow seminal root angle and a greater number of seminal roots are considered fundamental characteristics for early selection for root architecture in wheat breeding programs (Manschadi et al. 2008; Wasson et al., 2012 and Christopher et al., 2013).

Macaferri et al. (2016) evaluating a population of recombinant durum wheat seedling lines derived from the Colosseo cultivar not adapted to the arid conditions of the Mediterranean, and the Loyd cultivar adapted to drought conditions, in polycarbonate plates lined with germination paper after 9 days obtained a variation in the angle of the first pair of seminal roots from 48º to 147º, the Colosseo cultivar presented an angle of the first pair of seminal roots 42.0% wider than the adapted Loyd cultivar. Group 2 included 7 genotypes, with 3 cultivars recommended for sowing in adaptation region 4 under irrigation and 4 lines from the breeding program for adaptation region 3 (humid region). The angle between the first pair of seminal roots in group 2 varied from 84.39º to 103.61º, classified as a wide angle of the first pair of seminal roots.

Group 3 included 2 lines from the breeding program for adaptation region 4 (hot and dry) and the water deficit-tolerant cultivar BR18. The angle between the first pair of seminal roots in group 3 varied from 40.00º to 59.47º, classified as a narrow angle of the first pair of seminal roots. Group 4 clustered the control, cultivar BRS 404 adapted to water-restricted conditions, and two lines from the breeding program for adaptation region 4 (hot and dry region) that possess the gene of sunflower drought tolerance, Cadenza_HB4® x BRS_264 and Cadenza_HB4® x BRS_404. The genotypes presented angles between the first pair of seminal roots of 54.73º, 57.26º, and 58.37º, respectively, classified as a narrow angle of the first pair of seminal roots and plant adaptation field scores ranging from 7.76 to 8.01.

Group 5 included 1 line from the breeding program for adaptation region 4 (hot and dry region) classified as having an intermediate angle of the first pair of seminal roots (68.39º). Group 6 included 1 cultivar from the breeding program for adaptation region 3 (humid region) classified as having a wide angle of the first pair of seminal roots (105.07º). Group 7 included 1 line from the breeding program for adaptation region 3 (humid region) classified as having a wide angle of the first pair of seminal roots (87.63º). Group 8 included 1 cultivar recommended for irrigated planting in adaptation region 4, classified as having a wide angle of the first pair of seminal roots (84.40º).

Richard et al. (2015), evaluating a panel of wheat seedlings from different drought-tolerant adaptation regions using germination paper bags, observed after 10 days a variation in the angle of the first pair of seminal roots from 84.00º to 108.10º and an average of 109.70º. Cané et al. (2014), evaluating a panel of 183 elite durum wheat cultivars on polycarbonate plates covered with germination paper, after 10 days, observed a variation in the angle of the first pair of seminal roots from 48º to 147º with an average of 100º and seminal root length varying from 13.8 cm to 32.9 cm with an average of 21.10 cm. For the number of seminal roots, they obtained an average of 5.18 roots with a variation of 4.01 to 6.00 roots. The dendrogram obtained by the UPGMA method, from dissimilarity measurements between 30 wheat accessions, based on Euclidean distance for the angle of the first pair of seminal roots, root length and root number, grouped the genotypes into 8 dissimilarity groups (Figure 3).

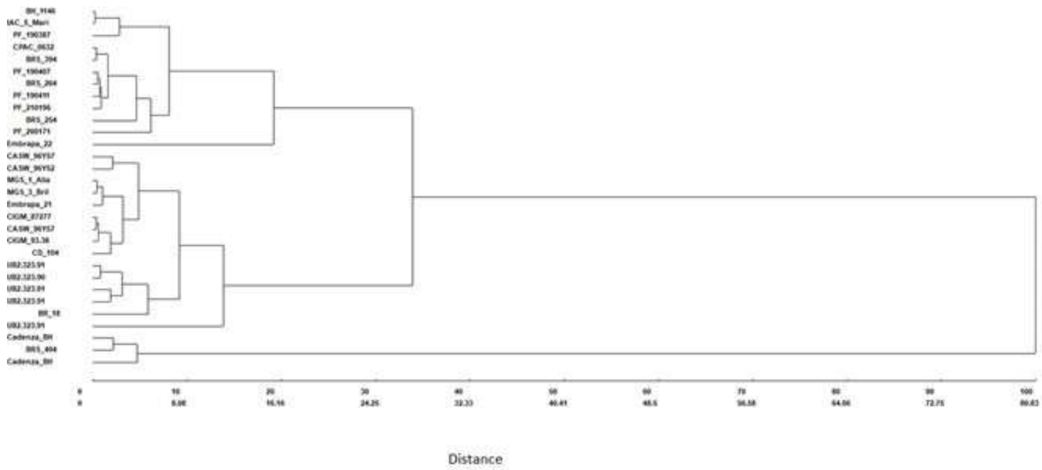

Figure 3. Dendrogram obtained by the UPGMA method, from dissimilarity measurements between 30 wheat accessions, based on Euclidean distance for the angle of the first pair of seminal roots, root length, root number and Plant Note Passo Fundo, 2025.

Results of root architecture characteristics from the present study suggest that the majority of cultivars and lines recommended for rainfed sowing at region 4 of adaptation were clustered at group 1 and group 4 and presented a narrower angle than genotypes recommended for region 4 irrigated and region 3 humid clustered at group 2, which presented wider angle values. There is consistency between the adaptation region of the wheat cultivars evaluated in this study and the cluster analysis based on root architecture characteristics. The cultivars and the lines recommended for the rainfed hot and dry region and the introduced lines from CYMMIT, when evaluated under field conditions in the Cerrado region of Uberaba, MG, showed a plant adaptation field score greater than 5 and an intermediate to narrow angle between the first pair of seminal roots. Conversely, cultivars recommended for irrigated cultivation and lines recommended for the humid region showed a plant adaptation field score of less than 5 and a wide angle between the first pair of seminal roots.

Although wheat breeders have indirectly selected for root architecture where selection pressure for environmental conditions is frequent, this is not the only characteristic affecting drought adaptation. Drought adaptation involves the interaction of several characteristics related to water use as well as physiological processes. For adaptation to various regions, breeders target characteristics of phenology, transpiration efficiency, canopy temperature reduction, and tillering reduction (Richard et al., 2015). Most of the genotypes clustered in group 1, that is, cultivars and lines adapted to drought conditions, presented an intermediate and narrow angle between the first pair of seminal roots, contrasting, for example, with the genotypes of group 2 adapted to region 3 (humid) or to irrigated cultivation, which presented a wide angle.

While the architecture of a deep root system can be important for adaptation to drought conditions and largely depends on the moisture maintained in the deeper layers of the soil, this characteristic may be less advantageous in other environments, such as more humid environments where rainfall is more frequent during the crop's growing season or under irrigation.

The angle of the first pair of seminal roots being narrow typically indicates a deeper root system and allows plants to explore water from the deeper layers of the subsoil (Wassson et al., 2012; Manshadi et al., 2008). Wheat cultivars with a narrow distribution of lateral roots and a greater number of roots can access greater soil moisture at depth in the soil profile, particularly during flowering and grain filling periods when there is greater demand by the plant (Manschadi et al., 2008). Wheat cultivars with a narrow angle between the first two seminal roots tend to have deeper roots and greater tolerance to water-deficient conditions. This germplasm can be used for future breeding work, allowing the selection of specific root types. Knowledge of the seminal root angle in different adaptation groups can be used to design ideotypes better adapted to contrasting environments such as irrigation regimes, drought, soil toxicity, or nutrient availability (El Hassouni et al 2018).

The suspended folder method allowed differentiation between the angle of the first seminal roots. The method showed 100% utilization of the repetitions because all the evaluated seedlings that were visible could be evaluated for root angle, being superior to soil cultivation methods where there is difficulty in differentiating the roots from the soil and it is frequent for the roots to be hidden by the soil, resulting in the inviability of repetitions (Richard, 2015 and El Hassouni et al., 2018). The method is efficient for identifying genetic variability and can be used to select parental genotypes with desirable characteristics in crosses. The method has the potential to accelerate genetic gain for drought tolerance in breeding programs. The technique allows for multiple evaluation cycles, making it suitable for use in "speed breeding" systems, where temperature and light control result in rapid plant growth.

Selection for seminal root architecture traits can also be easily integrated with other phenotypic selection methods, such as adult plant resistance, rust resistance, and grain dormancy for pre-harvest sprouting tolerance.

CONCLUSION

The genotypes BRS 404, Cadenza HB4® xBRS 264, Cadenza HB4® xBRS 404, BR_18, UB2.323.916, UB2.323.907, UB2.323.917, UB2.323.919 with a narrow angle between the first pair of seminal roots and a plant field score above 5 were selected as potential for planting under water-restricted environmental conditions. The suspended file folder method was efficient for observing the characteristics of the angle between the first two seminal roots, length, and number of roots, allowing for the discrimination of genotypes. Therefore, it is suitable for high-throughput, large-scale screening of root architecture features.

ACKNOWLEDGMENTS
The authors express gratitude to the National Council for Scientific and Technological Development (CNPq) for its financial support of the research under the project number 405115/2021-0..

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES

Bengough, A.G.; Gordon D.C.; Al-Menaie H.; Ellis R.P.; Allan D., Keith R.; Thomas W.T.B.; Forster B.P. (2004) Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant and Soil, 262:63 70. Available at [https://doi.org/10.1023/B:PLSO.0000037022.95485.72]

Canè, M.A.; Maccaferri, M.; Nazemi, G.; Salvi, S.; Francia, R.; Colalongo, C.; Tuberosa, R. (2014) Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. Mol Breed. Available at [4:1629-1645. https://doi.org/10.1007/s11032-014-0183-2]

Christopher, J.; Christopher, M.; Jennings, R.; Jones, S.; Fletcher, S.; Borrell, A.; Manschadi, A.M.; Jordan, D.; Mace, E.; Hammer, G. (2013) QTL for root angle and number in a population developed from bread wheats (Triticum aestivum) with contrasting adaptation to water-limited environments. Theor Appl Genet, 126:1563–1574. Available at [https://doi.org/10.1007/s00122-013-2064-6]

CONAB – Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira de Grãos: safra 2024/25: 11º levantamento, v. 12, n. 11, p. 1-102, agosto. 2025. Brasília: Conab, jan. 2025. Available at [chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.gov.br/conab/pt-br/atuacao/informacoes-agropecuarias/safras/safra-de-graos/boletim-da-safra-de-graos/11o-levantamento-safra-2024-25/e-book_boletim-de-safras-11o-levantamento-2025.pdf] accessed 04 November 2025.

Cruz, C. D. (2001) Programa GENES (versão Windows): aplicativo computacional em genética e estatística. Viçosa, MG: Universidade Federal de Viçosa. 648 p available at [https://doi.org/10.1590/S1415-47571998000100022] Acessed 4 November 2025.

Cruz, C. D.; Regazzi, A. J.; Carneiro, P. C. S. (2014) Modelos biométricos aplicados ao melhoramento genético. Viçosa, MG: Universidade Federal de Viçosa, v. 1, 668 p.

Cunha, G. R.; Scheeren, P. L.; Pasinato, A.; Pires, J. L. F.; Caierão, E.; Só E Silva, M.; Dotto, S. R.; Campos, L. A. C.; Castro, R. L.; Felício, J. C.; Franco, F. A.; Marchioro, V. S.; Riede, C. R.; Rosa Filho, O.; Tonon, V. D.; Svoboda, L. H. (2006) Regiões de adaptação para trigo no Brasil. Passo Fundo: Embrapa Trigo;. Circular Técnica nº 20. 35 p. Available at [https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/852170/1/pci20.pdf] Acessed 04 November 2025.

De Dorlodot, S.; Forster, B.; Pagès, L.; Price, A.; Tuberosa, R.; Draye, X. (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci, 12:474–481. Available at [https://doi.org/10.1016/j.tplants.2007.08.012]

El Hassouni, K., Alahmad, S., Belkadi, B., Filali-Maltouf, A., Hickey, L. T., & Bassi, F. M. (2018). Root system architecture and its association with yield under different water regimes in durum wheat. Crop Science, 58:96–106. Available at [https://doi.org/10.2135/cropsci2017.05.0297]

Hund, A.; Trachsel, S.; Stamp, P. (2009) Growth of axile and lateral roots of maize: I development of a phenotying platform. Plant and Soil, The Hague, 325:335-349. Available at [https://doi.org/10.1007/s11104-009-9984-2]

Lobet G, Draye X. (2013) Novel scanning procedure enabling the vectorization of entire rhizotron-grown root systems. Plant Methods, 9:1. Available at [https://doi:10.1186/1746-4811-9-1]

Lopes MS, Reynolds MP. (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct Plant Biol., 37: 147–56. Available at [https://doi.org/10.1186/1746-4811-9-1]

Maccaferri, M.; Sanguineti, M.C.; Corneti, S.; Ortega, J.L.; Salem, M.B.; Bort, J.; Deambrogio, E.; Del Moral, L.F.; Demontis, A.; El-Ahmed, A.; Maalouf, F.; Machlab, H.; Martos, V.; Moragues, M.; Motawaj, J.; Nachit, M.; Nserallah, N.; Ouabbou, H.; Royo, C.; Slama, A.; Tuberosa, R. (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (Triticum durum Desf.) across a wide range of water availability. Genetics, 1:489–511. Available at [https://doi.org/10.1534/genetics.107.079841]

Mairhofer S, Zappala S, Tracy S, Sturrock C, Bennett MJ, Mooney SJ, et al. (2013) Recovering complete plant root system architectures from soil via X-ray mu-computed tomography. Plant Methods. 9:8. Available at [https://doi.org/10.1186/1746-4811-9-8]

Manschadi A, Hammer G, Christopher J, DeVoil P. (2008) Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (Triticum aestivum L.). Plant Soil. 303:115–29. Available at [https://doi.org/10.1007/s11104-007-9462-1]

Reynolds, M., Dreccer, F.; Trethowan, R. (2007) Drought-adaptive traits derived from wheat wild relatives and landraces. J Exp Bot, 58:177–186. Available at [https://doi.org/10.1093/jxb/erl118]

Richard C AI; Hickey,LT; Fletcher S; Jennings R; Chenu, K.; Christopher JT (2015) High-throughput phenotyping of seminal root traits in wheat. Plant Methods, 11:1-11. Available at [https://doi.org/10.1186/s13007-015-0055-9]

Sanguineti, M. C., Li, S., Maccaferri, M., Corneti, S., Rotondo, F., Chiari, T., & Tuberosa, R. (2007) Genetic dissection of seminal root architecture in elite durum wheat germplasm. Annals of Applied Biology, 151:291–305. Available at [https://doi.org/10.1111/j.1744-7348.2007.00199.x]

Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SVS, Rebetzke GJ, et al. (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot., 63:3485–98. Available at [https://doi.org/10.1093/jxb/ers111]