

Improvement of common bean root system phenotyping for identification of superior genotypes

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ABSTRACT. The development of common bean plants with an improved root system can be a strategy for water and nutrient absorption in limiting environmental conditions. The objective of this study was to understand the influence of root phenotyping methods and phenological stages of evaluation on the selection of common bean genotypes for a highly branched root system. In the 2021/22 growing season, this study was initiated with 36 field treatments, consisting of the combination of three genotypes, two parents (Mesoamerican and Andean gene pools) and one progeny, two methods of root phenotyping (Shovelomics and WinRHIZO) and six growth stages (R_{1-6} : four and eight trifoliate leaves, flower bud, full flowering, pod formation and grain filling). The field treatments were randomized in a simple lattice design. Five plants from each experimental unit were evaluated, considering the genotype and phenotyping methods in each developmental stage. The genotype x method x stage interaction was significant. The partitioning of the simple effects of the factors indicated that the root system variables of the parents could be distinguished from those of the progeny at R_6 . At this stage, the recommended phenotyping method differs

according to the genetic origin of the genotypes. Plotting of the standardized canonical scores for the triple interaction showed that the Shovelomics and WinRHIZO phenotyping methods are adequate for the Andean and Mesoamerican genotypes, respectively, in view of their high scores with high discriminative power, allowing treatment discrimination. Specific phenotyping methods were indicated for Mesoamerican *versus* Andean genotypes in view of the root development trait intrinsic to each gene pool. We conclude that improving root phenotyping for the development of cultivars with a finely branched root system is a useful strategy to maintain common bean yields in environments under stressful conditions.

Key words: *Phaseolus vulgaris*; Andean gene pool; Mesoamerican gene pool; WinRHIZO; Shovelomics

INTRODUCTION

Most yield-related agronomic traits of common bean (*Phaseolus vulgaris*) are controlled by many genes, and favorable alleles for the development of superior genotypes can be found in different lines (De Paula et al., 2019). In view of the requirements for industrial food production, breeding programs have focused on improving above-ground traits, which are easy to measure and select among candidate genotypes (Sofi et al., 2021).

Although the valuable efforts of common bean breeders in the selection of improved genotypes have been successful, approximately 60% of the bean-producing regions in the world are affected by water scarcity (Wu et al., 2021a). This recurring situation means that the genetic yield potential of the crop is not fully exploited, and grain yield losses can exceed 80% (Mukankusi et al., 2019; Jochua et al., 2020). Due to this fact, the genetic variation between fixed and segregating populations in the expression of agronomic traits must be better understood, e.g., with regard to the root system, which is essential for the tolerance of plants grown under hostile or limiting conditions (Prince et al., 2019; Ribeiro et al., 2019). To develop this understanding, phenotyping for evaluations and the determination of variables of the plant root system are required. The different phenotypes of the root system play a key role in the adaptation to restricted environments and are doubtlessly traits to be incorporated in crop breeding (Burrige et al., 2020).

The development of cultivars with improved root traits is relatively difficult, due to the quantitative inheritance of traits associated with the root system and the pronounced effect of the environment factor on this trait, which can mask the selection of the best genotypes (Mir et al., 2012). In addition, another difficulty is that phenotyping the root system of genotypes in the field is an arduous and complex process (Li et al., 2017). At sampling, the root system may be damaged, causing root loss (Marshall et al., 2016; Sofi et al., 2021). Studies focused on high-throughput phenotyping have addressed the use of techniques that allow an efficient detection of the best progenies in breeding programs. The identification of genotypes with candidate genes for the biological development of the root system can be facilitated with high-throughput phenotyping techniques (Araus and Cairns, 2014; Prince et al., 2019).

This issue of phenotyping the root system in the field raises some questions. Can the phenotyping method breeders use accurately quantify the common bean root system? Can the currently used phenotyping methods comparatively discriminate fixed populations from segregating populations for root system characteristics? Is the physiological age of the plants (developmental stage) at evaluation adequate in combination with the phenotyping method of the root system? To quantify root system variables, plant breeders have already used some methods acknowledged in the literature, e.g., “Shovelomics” and “WinRHIZO” (Trachsel et al., 2011; BurrIDGE et al., 2016; Blaser et al., 2020). With regard to the physiological age, evaluations have been performed at the most critical points of crop development, namely at flower bud emission (stage R₅), full flowering (R₆) and pod filling (R₈), (BurrIDGE et al., 2016; Galvão et al., 2019; Wu et al., 2021b). In previous studies of our research group, a significant interaction between the phenotyping method and growth stage has already been observed. The interpretation of this information suggested that plant breeders should use a greater number of phenotyping stages, whereas the inclusion of more root evaluation methods in breeding programs might be a drawback, in view of the difficulties of field evaluations. Thus, determining a phenotyping method capable of differentiating fixed genotypes from segregating ones, at a given development stage will help breeders in choosing genotypes with developed root system traits, as it facilitates field evaluations.

Thus, the purpose of this study was to examine the influence of phenotyping methods and phenological stages on the discrimination of different common bean genotypes to optimize selection of genotypes with a more finely branched root system.

MATERIAL AND METHODS

The experiment was carried out in the field in the district of Lages, Santa Catarina, Brazil, in an experimental area of the Centro de Ciências Agroveterinárias (CAV), (27° 47'S and 50° 18'W) at 950 m asl. that belongs to the Santa Catarina State University (UDESC). The mean air temperature is 16 °C and average annual rainfall 1441 mm. The soil of the experimental area is a Cambissolo Húmico Alumínico Léptico, with a pH in water of 5.9; organic matter content of 3.2%; 3.1 mg P/dm³ and 124.0 mg K/dm³. Phosphorus and potassium contents were determined by the Mehlich-1 method (Cqfs-RS/SC, 2016). Climatic data of the experimental period were obtained from the NASA/POWER (Prediction of Worldwide Energy Resources) database (Sparks, 2018).

Studies carried out by our research group to identify a more appropriate method of root phenotyping in the common bean breeding program have been going on since the 2018/19 growing season, when 30 common bean genotypes were grown in the field (seven lines, nine F₁ populations and seven segregating F₂ and F₃ populations). In the tests with these fixed and segregating populations, no better phenotyping method for the evaluation of the root system could be identified, as similarly occurred in the 2019/20 growing season. In an attempt to circumvent this problem, in the 2020/21 growing season, experiments were implemented at two locations in the state of Santa Catarina. In these trials, three developmental stages were considered for root system phenotyping. Results indicated that the greatest variation in root system expression was caused by the interaction between the phenotyping method and phenological growth stages (method x stage interaction significant), with little relevance of the locations.

To explore this effect, 36 field treatments were installed in the 2021/22 growing season. These consisted of the combination of three experimental factors (genotypes, root phenotyping methods and phenological growth stages). The genotype factor (structured specific qualitative) consisted of two lines (parents BAF07 and BRS Embaixador) and one progeny in the F_6 generation, derived from the cross of the parents (BAF07 x BRS Embaixador F_6). This progeny was chosen for its superior performance in some root and shoot traits observed in previous trials. The genotypes used in this study had been evaluated for root system characteristics since 2016 (Cerutti et al., 2020; Grigolo et al., 2021). Parent BAF07 belongs to the black commercial group and to the Mesoamerican gene pool (1000-seed weight of 200 - 250g, seeds with phaseolin “S” and plants with type II and III growth habits). Parent BRS Embaixador belongs to the commercial colored group and the Andean gene pool (300 -500 g/1000 seeds, type “T” phaseolin and plants with type I growth habit (Nienhuis and Singh, 1988). The phenotypic performance of the progeny was compared with the parent mean.

The phenotyping method (specific unstructured qualitative factor) consisted of two levels, called “Shovelomics and WinRHIZO™”. Applications of these two systems for the evaluation of root variables of the most diverse crops (maize, wheat, canola, soybean, sugarcane and common bean) have already been described in the literature. The Shovelomics approach was developed by Pennsylvania State University researchers. It enables high-throughput phenotyping of plants in the field. The evaluation is performed by placing the plant roots in a template, where the variables of interest are measured (Trachsel et al., 2011). The WinRHIZO method consists of a scanning software, in this study operated with an Epson Expression 10,000 XL Scanner (Regent Instruments Canada Inc.). By this method, the plant roots are placed on the equipment and the software measures the variables quantitatively, automatically and simultaneously by means of an image generated by the system (Pornaro et al., 2017). The phenological growth stages comprised the third experimental factor (structured specific qualitative), with six levels, i.e., $V_{4.4}$ (four trifoliate leaves; $V_{4.8}$ (eight trifoliate leaves); R_5 (floral bud emission); R_6 (full flowering); R_7 (beginning of pod formation) and R_8 (pod filling) (Gepts and Fernández, 1982).

The randomized field treatments were arranged in a partially balanced simple lattice design (6 x 6), with two replications, resulting in a total of 72 experimental units. The use of lattice designs in the area of plant breeding is common, due to the high number of treatments to be evaluated. Under these conditions, the above design is more effective than that of complete randomized blocks, because by dividing the experimental area into more uniform blocks, the homogeneity within each block increases. In this experiment, the relative efficiency of the lattice design was 93.4% and, compared with the randomized complete block design, justified its use in the study (Gomez and Gomez, 1985). The experimental units (2 x 2 m) were spaced 1.0 m apart. After sowing on November 27, 2021, at a density of 13 seeds per meter (260,000 plants per hectare), the plants were harvested in the last stage on February 22, 2022 (87 days later). All management practices such as fertilization and weed, insect and disease control for the crop were applied as required, according to the regional technical recommendations for common bean (Fancelli and Neto, 2007; Cqfs-RS/SC, 2016).

Once the plants of the experimental plots assigned to each of the phenotyping methods reached the corresponding stages, the root system was phenotyped. By both methods, this step consisted of excavating the soil around the plant stem within a distance of

0.25 to 0.30 m and to a depth of 0.30 m, to lift out the soil / root clump. Five common bean plants were randomly collected from each experimental unit. Thereafter, the excess soil was removed to expose the roots, which were cleaned by immersing them in water containers with 5% neutral detergent (Trachsel et al., 2011). For the Shovelomics protocol, the following variables were measured: *i*) basal root angle (AG, °); *ii*) vertical root length (VL, cm); *iii*) horizontal root length/ left side (HLL, cm) and *iv*) horizontal root length/ right side (HLR, cm). By the WinRHIZO method, the variables *v*) total root length (TRL, cm); *vi*) projected root area (PRA, cm²); *vii*) root volume (RV, cm³) and *viii*) mean root diameter (DM, mm) were evaluated. As the response variables of the phenotyping methods are not the same and have different magnitudes, log y+1 transformation was applied for standardization, to meet multivariate normality (Yeater et al., 2015).

Based on the experimental factors of fixed effect and the variables evaluated in the experiment, the mathematical model $Y = XB + E$ was adopted, where: *Y* represents the matrix of the set of response variables; *X* the experimental design matrix considering fixed effect factors, i.e., the representation of the experimental field plots; *B* the matrix for the estimation of the treatment parameters and *E* represents the matrix of variances (diagonal direction) and residual covariances (above and below the diagonal), i.e., it is the matrix of experimental residue/error arising from the uncontrolled experimental conditions. When multivariate normality of more than one response variable has to be tested, one solution is to check it by means of standardized distances of the collected variables, based on quartiles, another is to use bi-dimensional scatter-plots, observing a tendency of the multivariate curve, or as a third possibility, a generalization of univariate normality tests can be used, based on asymmetry and kurtosis estimates (Yeater et al., 2015). In this way, the hypothesis of multivariate normality was tested by the Mardia test, based on asymmetry and kurtosis estimates (Mardia, 1970). After applying multivariate variation analysis, contrasts between mean vectors were tested to draw inferences about the simple effects of the experimental factors. For these analyses, software SAS (SAS OnDemands for Academics), the general linear model (GLM procedure) and MANOVA (multivariate analysis of variance) were used.

RESULTS AND DISCUSSION

The null hypothesis regarding multivariate normality was accepted at a significance level of 0.05 by the Mardia test ($p=0.2171$). In case of compliance with normality, several procedures can be applied for the analysis and treatment of multivariate data, according to the objective of each experiment (Cain et al., 2017). Based on the performance of the multivariate analysis of variance, Wilks' Lambda (λ) test indicated a significance level of 0.05 for the effect of triple interaction between the factors genotype x method x stage and for the interactions genotype x stage and method x stage (Table 1).

This triple interaction evidences the difference between the simple effects of the genotype x method interaction and the simple effects of the factor evaluation stage. This result shows that a phenotyping method ought to be combined with a specific developmental stage, for each genotype under study. This indicates that non-genetic or environmental factors (method and stage) had an important effect on root trait expression. The significance of the genotype x environment interaction has been reported in field root measurements (Jochua et al., 2020). These authors carried out experiments in contrasting

environments (Pennsylvania – USA and Chokwe – Mozambique), with 196 common bean accessions of the Andean and Mesoamerican groups and identified a strong environmental effect on common bean root development.

The correct phenotyping of root variables is fundamental for the development of common bean genotypes with improved root systems (Abenavoli et al., 2016). Thus, the identification of a method associated with a more suitable plant growth stage to carry out phenotyping will help plant breeders choose the best time to select the best genotypes. If the method and growth stage are adequate, it may be possible to distinguish fixed from their segregating populations based on root characteristics evaluated in the field. So far, this has not been successful throughout the course of the common bean breeding program conducted by our research group (De Melo et al., 2018; Cerutti et al., 2020; Grigolo et al., 2021).

Table 1. Multivariate analysis of variance based on Wilks' Lambda test (λ), for the effects of the experimental factors replication, blocks within replications, genotype, phenotyping methods (Shovelomics and WinRHIZO) and phenological stages (V_{4-4} ; V_{4-8} ; R_5 ; R_6 ; R_7 and R_8) in common bean, considering all response variables that were phenotyped.

Source of variation	Wilks' Lambda (λ)	Pr>F
Replication (R)	0.98	0.3470
R (Block)	0.85	0.1647
Genotype (G)	0.98	0.6188
Method (M)	0.01*	0.0001
Stage (St)	0.41*	0.0001
G*M	0.97	0.4774
G*St	0.76*	0.0001
M*E	0.20*	0.0001
G*M*St	0.75*	0.0001

$H_0: \mu_1 = \mu_2 = \dots = \mu_k$. * Significant at 0.05 probability by the F test.

An analysis of the effect of the genotype x method x stage interaction showed that the first two canonical discriminant functions captured, respectively, 60 and 24% of cumulative variation. In this situation, no discriminative power between the factors was observed for the variables horizontal length/right side (HLR) and mean root diameter (MD), with standardized canonical scores close to or equal to zero (-0.017 to -0.48). On the other hand, the variables basal root angle (AG), total root length (TRL), vertical root length (VL), projected root area (PRA), horizontal length/left side (HLL) and root volume (RV) contributed most to discriminate the experimental factors, with standardized canonical scores between 1.95 and 4.88.

The studies focused on root trait expression in common bean, with emphasis on understanding water stress tolerance, highlight that the variables root volume, total root length, mean root diameter and basal root angle are fundamental for the evaluation of genotypes in terms of drought tolerance (Kachiguma et al., 2021; Wu et al., 2021b). The study of WU et al. (2021b) stresses the importance of the variables root volume, diameter, length and angle to (assess) tolerance to water stress. In our study, all these variables mentioned in the literature were important to discriminate factors related to interaction, except for mean root diameter. Possible causes for the low contribution of this variable may be the negative correlation with root length, i.e., plants with greater root length have thinner and smaller roots, or the molding of the root system according to the soil it grows in.

Depending on the soil physical properties, the difficulty of plants to explore the soil layers is greater or smaller, which may affect root diameter.

The plotting of the standardized canonical coefficients showed that for the genotype x stage interaction the different levels of the stage factor induced variation in the levels of the genotype factor (Figure 1a). In this situation, the stages V_{4-8} , R_6 and R_8 were most effective in the discrimination of the genotypes. The variation in V_{4-8} and R_8 was significant at the level of 0.05 by the Wilks' Lambda (λ) test ($p=0.001$ and $p=0.0266$, respectively). The interaction between method x growth stage is shown in Figure 1b. The visualization of the canonical flow dispersion of the growth stages assessed by Shovelomics and WinRHIZO demonstrated that V_{4-8} , R_6 and R_8 were also effective to discriminate the factors and should possibly be considered for field phenotyping of root characteristics. At these stages, the canonical scores of both methods were positive and close to each other, indicating discriminatory power of the treatments. Positive canonical scores differentiate the treatments, whereas negative scores approach them. Determining the most appropriate time of evaluation of the root system is essential for researchers to eliminate the gap between an ideal evaluation of the root system and the conditions of phenotyping under cultivation conditions in the field (Falk et al., 2020).

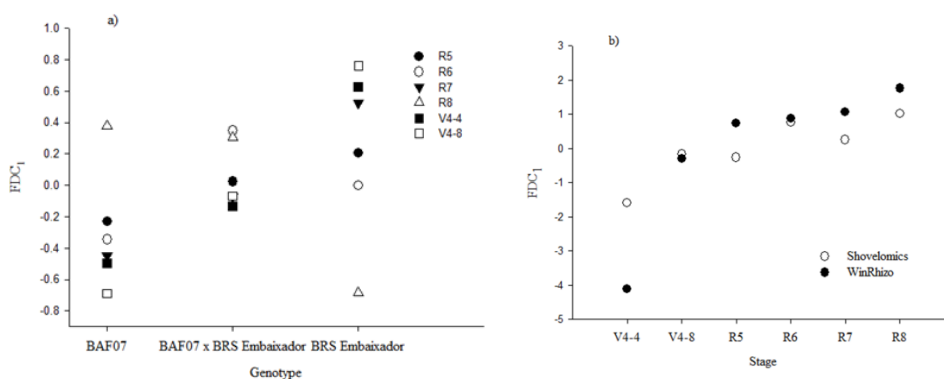


Figure 1. Dispersion of standardized canonical scores for the first canonical discriminant function (CDF), estimated for the interactions between: a) genotype (BAF07, BAF07 x BRS Embrapa, BRS Embrapa), and phenological stages (V_{4-4} ; V_{4-8} ; R_5 ; R_6 ; R_7 ; R_8) and b) phenotyping method (Shovelomics, WinRHIZO) and developmental stage in common bean. Positive canonical scores differentiate treatments.

The variation in growth stages of the genotypes may also be associated with oscillating weather conditions during the crop cycle (Figure 2). In the 2021/22 growing season, 266 mm cumulative rainfall was measured in the district of Lages.

This amount of rain is below the recommendation for the common bean (300 – 400 mm) (Sofi et al., 2018). The mean cumulative rainfall in the 2020/21 growing season was 490 mm, 224 mm more. This may have affected the physiological development of the common bean, causing changes in the cycle, with a direct influence on growth stages. On the other hand, for studies related to common bean roots, low water levels during the crop cycle are ideal. Importantly, the analogous behavior of segregating compared to fixed populations of common bean with regard to the root system remained constant over the years of evaluation, even in growing seasons with alterations in the plant-available water level.

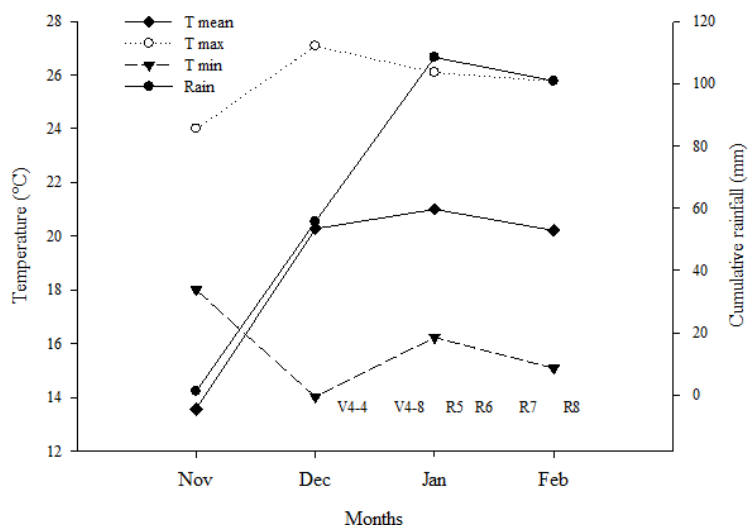


Figure 2. Representation of weather data: maximum, minimum, mean temperature (T max, T min, T mean, °C), cumulative rainfall (Rain, mm) and indication of time when evaluations were carried out, according to each phenological stage (V_{4.4}; V_{4.8}; R₅; R₆; R₇; R₈) proposed for the crop (2021/22) growing season; Source: NASA, 2022, obtained by A NASA POWER Global Meteorology, Surface Solar Energy and Climatology Data Client for R present in SPARKS, 2018.

To come to conclusions about the interaction between genotype x method x stage, the simple effects of genotype factors and phenotyping method were explored for each stage by the multivariate contrast technique. Contrasts one, two, five and six suggest that stages V_{4.4} and R₅ are not appropriate for root phenotyping (non-significant effects at $p \leq 0.05$), by Wilks' Lambda test (λ), (Table 2). These stages did not favor the distinction between fixed populations by either method (contrasts one and five) nor the distinction between fixed populations x progeny (contrasts two and six). This shows clearly that there is no advantage in phenotyping the roots at the beginning of crop development (V_{4.4}) or in the transition between the vegetative and the reproductive period (R₅). By phenotyping at stage V_{4.8}, the fixed populations of progenies can be discriminated (contrast four, $p=0.0173$) and consequently, the progenies could be differentiated and compared to their parents. In stages R₇ and R₈, differences were only detected between fixed populations.

At the reproductive stage (R₈), which involves pod formation and filling, differences between parents only were detectable (contrast 11). This difference may be associated with the genetic origin. Parent BAF07 is derived from the Mesoamerican gene pool, has small seeds (200 g/1000 seeds) and, mainly, an indeterminate growth habit. In plants with this growth habit, pod formation and filling can be extended, depending on the weather conditions of the growing cycle. Parent BRS Embaixador belongs to the Andean gene pool, with characteristically larger seeds (630g/1000 seeds) and a determinate growth habit (Nienhuis and Singh, 1988; Beebe et al., 2013; Polania et al., 2017). Regarding the growth habit, genotypes with indeterminate growth tend to develop a larger root system than those with determinate habit which have inherently reduced root growth and development (Velho et al., 2018).

Table 2. Multivariate contrasts based on the Wilks' Lambda test (λ), considering all response variables, for the simple effects of the experimental factors phenotyping methods (Shovelomics, WinRHIZO) and genotype for each of the phenological stages (V_{4.4} to R₈), in the common bean.

Contrast		λ	Pr > F
	V _{4.4}		
01) Methods vs. Parents		0.98	0.3492
02) Methods vs. (Parents x Progeny)		0.99	0.7722
	V _{4.8}		
03) Methods vs. Parents		0.97	0.0666
04) Methods vs. (Parents x Progeny)		0.96*	0.0173
	R ₅		
05) Methods vs. Parents		0.99	0.6629
06) Methods vs. (Parents x Progeny)		0.99	0.5842
	R ₆		
07) Methods vs. Parents		0.96*	0.0487
08) Methods vs. (Parents x Progeny)		0.96*	0.0243
	R ₇		
09) Methods vs. Parents		0.95*	0.0045
10) Methods vs. (Parents x Progeny)		0.99	0.9864
	R ₈		
11) Methods vs. Parents		0.94*	0.0029
12) Methods vs. (Parents x Progeny)		0.98	0.4302

* Significant at 0.05 probability by the *F* test.

In plants with indeterminate growth habit, the transition from the vegetative to the reproductive stage is gradual, and this can be a strategy to circumvent the effects of water stress, for example. This occurs by the continuous development of vegetative and reproductive structures, which make the plant more resistant to support a period of water stress. In general, four mechanisms of adaptation to water stress are observed in plants, namely: i) stress prevention; ii) stress tolerance; iii) escape and iv) stress recovery (Wu et al., 2021b).

As well as these stages with significant contrasts (V_{4.8}, R₇ and R₈), the full flowering stage (R₆) also obtained best results in the root evaluation of both fixed and segregating populations, which can be observed in contrasts seven and eight ($p=0.0487$ and 0.0243). This stage contributed to the discrimination of fixed from the segregating population for the set of root-related variables. Root phenotyping at this stage will possibly help plant breeders in the choice of superior genotypes during the stages of selection of segregating populations in the breeding program. A correct or highly reliable quantitative characterization of the root system is extremely relevant for the evaluation of a phenotype in the field (Narisetti et al., 2019). On this basis, detailed decisions are made and genotypes that meet the desired characteristics will be selected. (Assefa et al., 2013; Bery Mier Teran et al., 2019).

For the significant contrasts inherent to stage R₆, the greatest contributions of the variables to discriminate the factors were made by horizontal root length/left side and root volume (canonical coefficients 3.98 - 6.55; data not shown). In addition to these characteristics, basal root angle, root length and projected root area also contributed significantly (canonical coefficients 0.79, 0.65 and 0.33, respectively). Thus, these variables are very important and must be measured at root phenotyping, by both the Shovelomics and the WinRHIZO approach. Among the characteristics associated with the common bean

ideotype for the root system, the following are most relevant: small basal root angle, long length and major volume of the root system (BurrIDGE et al., 2020). Plants with a basal angle close to 90°, with a branched and voluminous root system will possibly be more apt to capture water resources in restricted environments.

A fact that may explain the significance of the contrasts in the full flowering stage (R_6) is related to a peculiar characteristic of the root system. Phenotypic plasticity is observed in the roots, defined as the ability of an organism to change its phenotype (or the expression of its characteristics) in response to the current environment to which it is exposed or where it is being cultivated. There are even reports that the plasticity of progenies can be affected by the way their parents adapted or shaped their phenotype in the environments where they were cultivated (Lorts et al., 2020). Due to the distinct and specific geographic adaptation of genotypes from the Mesoamerican compared to the Andean gene pool, variations in root system expression are common when these genotypes are cultivated in the most varied environments (Nienhuis and Singh, 1988; Ehdai et al., 2012; Strock et al., 2019; Schneider and Lynch, 2020). In this study involving genotypes from different gene pools, the levels of plasticity in the system differed, influenced by the phenotyping method and growth stage. The plasticity of the genotypes can also be an adaptive strategy to water deficit. Those with indeterminate growth habits are possibly more likely to tolerate and recover from water stress, while on the other hand, those with a determinate habit are associated with the ability to escape water stress (Velho et al., 2018).

In addition to the multivariate contrasts, the standardized canonical scores are presented for the two discriminant functions of the stages whose simple effects were significant ($V_{4,8}$, R_6 , R_7 and R_8), for the three genotypes and the two phenotyping methods (Figure 3). As can be seen, for all stages there was agreement between the genotype factor and the most appropriate phenotyping method (positive canonical scores on the right hand of each graph). The quantification of the root system of parent BAF07 (G_1) and progeny BAF07 x BRS Embaixador F_6 (G_3) was better when phenotyped by WinRHIZO (M_2), at all stages. Parent BRS Embaixador (G_2) was associated with the Shovelomics method (M_1) (positive canonical scores on the right hand of the graph). This consistent information for all growth stages indicates the reliability of the phenotyping methods, as they repeat the information throughout the crop cycle.

It can be expected that different phenotyping methods will be identified as best-suited for the parents BAF07 (Mesoamerican) and BRS Embaixador (Andean). Genotypes of the Mesoamerican pool have the feature of a deeply developed root system (basal root angle close to 90°), compared to genotypes of the Andean group (root system concentrated in the soil surface; basal root angle of 180°) (Beebe et al., 2013; Polania et al., 2016).

In this way, a more specific method to quantify the root system of each genotype group should be used. WinRHIZO is accurate when measuring variables that are hard to determine manually, such as root volume and mean root diameter (Walter et al., 2015; Blaser et al., 2020). Since Mesoamerican genotypes develop a deep root system, this method seems appropriate, for allowing a better quantification of the variables root volume and mean diameter. Progeny phenotyping was also associated with the WinRHIZO method. This may be related to the target characteristics of selection for this progeny in the previous segregating generations (F_2 to F_5), e.g., black tegument color and mainly indeterminate growth habit, resembling parent BAF07.

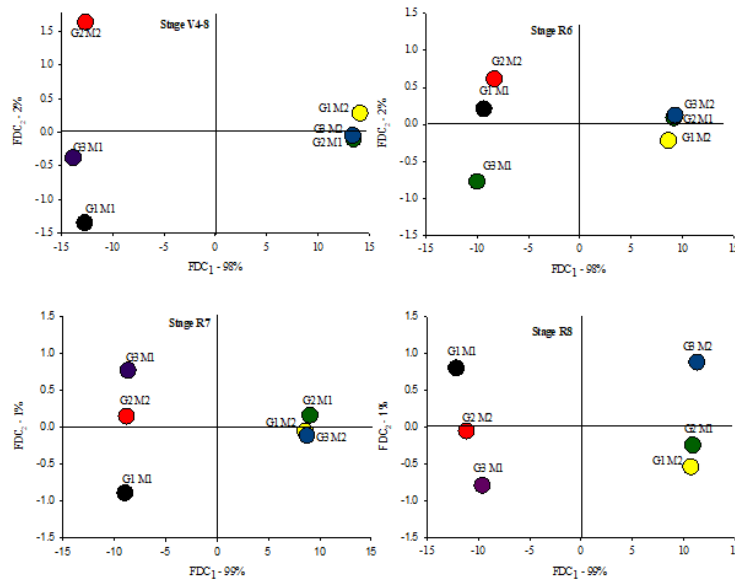


Figure 3. Dispersion of standardized canonical scores for the two canonical discriminant functions (CDF), estimated for the factors phenological growth stage (V₄₋₄; V₄₋₈; R₅; R₆; R₇; R₈), genotypes (G₁= BAF07; G₂=BRS Embaixador and G₃= BAF07 x BRS Embaixador F₆) and phenotyping methods (M₁= Shovelomics, M₂= WinRHIZO) in common bean. Positive canonical scores, on the right side of each graph, differentiate treatments.

For Andean genotypes, whose root development is concentrated in the upper soil layers, the Shovelomics approach may be more appropriate. This method is effective in high-throughput phenotyping in the field, since the evaluation of the root system after plant collection is simple. Thus, it is indicated for the variables basal root angle, vertical root length and horizontal root length/left side, previously defined as essential for evaluation in root breeding programs (Fenta et al., 2014).

When dealing with breeding for root-related and other characteristics, it is extremely important to discriminate the phenotypic performance of the resulting progenies in comparison with their parents. Since 2015, research on the root system has made no headway in the distinction between fixed *versus* segregating populations for root-related traits. These studies used renowned phenotyping methods at the proposed stages (R₆ and R₈), (Trachsel et al., 2011). Thus, the inclusion of a relatively new phenotyping method (WinRHIZO) and the expansion of growth stages in which phenotyping was performed was a viable alternative that circumvented the inconvenience of differentiating the genotypes. Thus, an advantage of this study is that relatively common phenotyping methods were used, by which a larger set of root variables can be exploited.

The findings of this study are important for common bean breeding programs, with a view to developing genotypes with an improved root system. The reason is that the task of selecting genotypes with an improved root system is still a challenge to be overcome by breeders (Hochholdinger et al., 2018). The integration of data of phenotyping methods and evaluation stages can contribute to this process and to a well-founded choice of common bean genotypes with root phenotypes adapted to cultivation in adverse environments, with limited water and mineral resources.

CONCLUSIONS

The use of more specific phenotyping methods for the genotypes under study made an adequate measurement of the root system possible. The root system of genotypes of the Mesoamerican pool was better quantified by the WinRHIZO method and that of the Andean pool by the Shovelomics approach. By an additional evaluation of the root system in the full flowering stage (R6), the performance of fixed genotypes could be distinguished from and compared with the progeny. The association between phenotyping method and developmental stage can help plant breeders make sound choices of common bean genotypes with improved root traits, which will contribute to sustain plant growth and development in water- and nutrient-stressed environments.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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