

Root characterization of bean genotypes (*Phaseolus vulgaris*) under drought stress

A.Teixeira*, D.A. da Silva*, J.G.R. Gonçalves, J.A. F. Esteves,
S.A.M. Carbonell and A.F. Chiorato

Instituto Agronômico (IAC), Centro de Grãos e Fibras, SP, Brasil

*These authors contributed equally

Corresponding author: D.A. da Silva
E-mail: daiagrouel2002@hotmail.com

Genet. Mol. Res. 18 (1): gmr18086
Received July 25, 2018
Accepted December 18, 2018
Published January 25, 2019
DOI <http://dx.doi.org/10.4238/gmr18086>

ABSTRACT The morpho-agronomic characterization of gene banks is a key step in enabling breeding programs to efficiently exploit genetic resources. Several studies have evaluated the root system traits of the common bean to develop genotypes better adapted to drought. We examined the root systems of 1000 common bean accessions, from the Agronomic Institute of Campinas gene bank to: count the number of whorls and basal roots, estimate the divergence between accessions and evaluate the 47 most divergent genotypes under drought. The average numbers of whorl and basal roots for the 1,000 accessions were 2.07 and 8.09, respectively. Seven different clusters were identified using the Mahalanobis genetic divergence analysis and the Tocher optimization method, from which the 47 most divergent genotypes were selected. Both genotype and water treatments, in the pre-blooming period, significantly affected root length, root surface area, root volume, number of pods, seeds per plant and grain yield. However, only water treatment significantly affected stomatal conductance, with water treatment x genotype interaction only having a significant effect on this characteristic. Water deficit reduced the average grain yield by 52%; the most promising genotypes under water deficit considering both root growth and grain yield were: RAI 76, 56 Retinto Santa Rosa, SER 28, Bayo, IAC Una, IAC Bico de Ouro and 12-D.

Key words: germplasm characterization; root system; water deficit; common bean

INTRODUCTION

The study of root systems helps to develop drought-tolerant cultivars to provide effective alternatives (Singh, 2007) for areas under water stress. The availability of cultivars adapted to prolonged drought periods is essential for maintaining agricultural production, especially since climate change and global population increase are converging to challenge crop production systems to meet global food demand (McClean et al. 2011).

Genetic resources provide the basis for selection and improvement through plant breeding to ensure food security for a rapidly rising population. Conservation and characterization of morphagronomic traits are important in crop improvement programs all over the world (Upadhyaya et al. 2008).

The common bean has wide genetic variability in root traits. These genotypic differences are seen in root mass and architecture (Rubio & Lynch 2007), gravitropism of basal roots (Liao et al. 2001), number of basal roots and whorls (Viera et al. 2008) and amount of root hairs (Viera et al. 2007). Various multivariate methods can be applied to study genetic diversity, among them the structuring of the genetic dissimilarity matrix employing the Euclidean distance, Mahalanobis generalized distance (D^2) and coincidence indices. These multivariate analysis methods have been used to estimate the genetic diversity in the common bean (Chiorato et al. 2005; Ceolin et al. 2007).

Various researchers, including O'Toole et al. (1977), Cornic & Briantais (1990), Pimentel et al. (1999a), Pimentel et al. (1999b); Blum (1997), Molina et al. (2001), Terán & Singh (2002), Santos et al. (2004), Oliveira et al. (2005), Santos et al. (2006a), Santos et al. (2006b), Beebe et al. (2008) and Polania et al. (2009), have investigated the mechanisms related to drought tolerance to validate methodologies that help identify drought-tolerant genotypes. Blum (1997) recommends evaluating traits correlated with yield. Polania et al. (2009) recommends evaluating the root system, and O'Toole et al. (1977) and Oliveira et al. (2005) suggested stomatal control as a good indicator of drought.

We examined the root systems of 1000 common bean accessions for the numbers of whorls and basal roots, the divergence among them was estimated and 47 of these genotypes were then selected for investigation under drought stress.

MATERIAL AND METHODS

One thousand common bean accessions from the Agronomic Institute (IAC) gene bank were evaluated to determine the average numbers of whorls and basal roots. The experimental design was completely randomized with three replications. For each genotype, five seeds were germinated between two sheets of germination paper soaked in distilled water at a ratio of 2.5 times the mass of the dry paper. Germination rolls were transferred to a germination chamber at temperature of $25 \pm 3^\circ\text{C}$ and after five days, the seedlings were removed to count the whorls and basal roots.

The divergence among genotypes for the number of whorls and basal roots traits was determined by the generalized Mahalanobis distance (Mahalanobis, 1936) taking into account the residual variance and covariance between measured traits and the possibility of correlation between them. The accessions were clustered by the Tocher optimization method using, as dissimilarity, the generalized Mahalanobis distance. The Tocher algorithm identified the different clusters (dissimilarity clusters) by grouping the

accessions by degree of similarity or dissimilarity. Both analyses were performed using the Genes software (Cruz 2006) and the figures made with R software (R Development Core Team, 2012).

After divergence analysis and clustering of the 1000 accessions, 47 of the most divergent were selected; this selection included members from each of the clusters. The selection sought to identify genotypes of different commercial clusters, with different numbers of whorls and basal roots. To this end, a number of genotypes were selected by seed coat color: 12 black, 12 red, 9 cream, 4 carioca, 3 rose, 2 yellow, 1 green, 1 brown, 1 mulatto, 1 cream brindled with black and 1 white.

After germination, one seedling was transplanted per rhizotron using, as substrate, a mixture of clayey Red Eutrophic Oxisol and sand, at a 3: 1 ratio. Soil chemical conditions were: pH (5.7); P (80 mg.dm⁻³); S (12 mg.dm⁻³); K (3 mmol_c.dm⁻³); Ca (67 mmol_c.dm⁻³); Mg (19 mmol_c.dm⁻³); sum of bases (89 mg.dm⁻³); H+Al (38 mg.dm⁻³); Cation exchange capacity (127 mmol_c.dm⁻³) and base saturation (70%). An initial soil correction and fertilization was considered unnecessary. Twenty days after transplantation a cover fertilization was applied, consisting of 150 kg ha⁻¹ N with urea.

The rhizotrons, shaped as a half cylinder with 38-liter capacity (0.2m x 0.4m x 0.6m), were placed in wooden structures that raised them one meter above the ground and inclined at a 45° angle inside the greenhouse. A drip system irrigated the plants twice daily for one minute at a rate of 0.225 L min⁻¹.

The experimental design was a split plot with two plots each of 47 subplots and three replications. The plots reflected two water treatments, i.e., irrigation and drought in the pre-blooming (R5 Stage) according to Gonçalves et al. (2015). Each subplot had the 47 selected genotypes (Table 1). The water restriction treatment, “drought stress”, started at the R5 stage and lasted 15 days, without irrigation, at which time the degree of water deficit was determined by soil humidity sensors and Watermark® meter (200SS model) showing a soil matrix potential of around -199 kPa, which was considered as dry soil at a depth of 0.40 m. The stomatal conductance was measured after water deficit imposition (15th day) in a dynamic equilibrium state, using a porometer (AP4, Delta T Devices), to ensure drought effectiveness. Readings were performed between 9 am and midday on the abaxial faces of fully expanded leaves from the mid-point of the plant stem.

After physiological maturity, the roots were removed from the ground, washed in water and, kept in 20% ethanol. The root images of each plant were obtained using the LA2400 scanner (EPSON) and root traits were determined by the WinRHIZO® software (Regent Instruments Inc., Quebec, Canada). The root traits evaluated were: root surface area in cm² (SA), total length in cm (RL), average diameter in mm (RD) and, total volume in cm³ (RV). The intensity index of water stress was calculated with the following formula:

$$IIE = 1 - \frac{Xd.h}{Xi} \quad (Eq 1)$$

where: *Xd.h* is the average yield of all genotypes under drought, and *Xi* is the average yield of all genotypes under irrigation (CIAT, 2012).

Table 1 – Record number, genotype, commercial cluster and origin of 47 common bean genotypes selected for the drought trial.

	Record ¹	Genotype	Commercial	Origin ²	N ^o	Reco	Genotype	Commerci	Origin
1	33	Jalo	Yellow	CIAT	25	613	D. Calima	Cream	CIAT
2	40	Retinto Santa Rosa	Red	CIAT	26	633	RG 342 CH/60	Black	CIAT
3	84	LEG 50600	Black	CIAT	27	672	Iapar 81	Cream	IAPAR
4	89	Venezuela 350	Black	Venezuela	28	699	Bayo	Cream	UFV
5	95	2227 Mulato Branco	Cream	UFV	29	707	IAC Una	Black	IAC
6	101	Plant Type ≠ 1	White	CIAT	30	712	IAC Bico de ouro	Cream	IAC
7	203	Rosado 14- Mulatinho	Mulatto	UFV	31	729	L. 476-4	Cream	IAC
8	216	91/71-212	Black	CIAT	32	742	L. 309-1	Cream	IAC
9	221	12-D	Black	CIAT	33	832	Pérola	Carioca	EMBRAPA
10	246	56 Retinto Santa Rosa	Black	IAC	34	844	Pintado Rajado	Cream	CIAT
11	288	Rosinha	Rose	IAC	35	1139	Rubi	Carioca	IAC
12	325	Rosinha G2	Rose	IAC	36	1180	IAC Alvorada	Carioca	IAC
13	457	Cavalo Amarelo	Yellow	UFV	37	1181	IAC Galante	Rose	IAC
14	474	73 Vul 6686	Black	IAC	38	1183	IAC Diplomata	Black	IAC
15	481	Carioca MG	Carioca	UFV	39	1698	IAC Boreal	Red	IAC
16	485	Dom Timóteo	Red	CIAT	40	1703	IAC Jabola	Green	IAC
17	499	CF 830186 Vermelho	Red	EMBRAPA	41	1979	ALB 213	Red	CIAT
18	501	38883 Preto	Black	CIAT	42	1996	BFS 39	Red	CIAT
19	514	CF 820510 Santa Rosa	Rose	EMBRAPA	43	2000	BFS 60	Red	CIAT
20	531	PI 310724	Black	CIAT	44	2096	SCR 17	Red	CIAT
21	561	Lagartixa Precoce	Cream/Black	CIAT	45	2118	SER 28	Red	CIAT
22	575	RAI 76	Black	CIAT	46	2132	SER 210	Red	CIAT
23	592	Cal 153	Red	CIAT	47	2143	SMC 9	Cream	CIAT
24	593	EMP 407	Carioca	EMBRAPA					

¹Record number in the catalogue of the common bean gene bank in the Instituto Agronômico-IAC.

²Genotype origin: CIAT (International Center for Tropical Agriculture), IAC (Instituto Agronômico de Campinas), IAPAR (Instituto Agronômico do Paraná), EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), UFV (Universidade Federal de Viçosa).

At the end of the maturation period, the root dry mass in grams (RDM), number of pods per plant (NPP), number of seeds per plant (NSP), and grain yield in grams (Y) were determined. The data was submitted to variance analysis. Pearson correlation analysis was used to correlate the root traits with the yield compounds under water restriction for the following traits: stomatal conductance (SC); root length (RL); root surface area (RSA); root volume (RV); average root diameter (RD); root dry mass (RDM); number of seeds per plant (NSP); number of pods per plant (NPP); grain yield (Y); number of whorls (NW), and the number of basal roots (NBR).

Statistical analyses were performed by the Genes (Cruz 2006) and R software (R Development Core Team, 2012).

RESULTS AND DISCUSSION

There was significant individual variance in the number of whorls and basal roots between genotypes. Genetic variation coefficients were 13.12 and 13.66% for whorls and basal roots, respectively, indicating genetic variability among genotypes. This result, associated with a coefficient determination greater than 95% for the two traits, indicated that they were suitable for the Mahalanobis genetic dissimilarity analysis to identify any divergent clusters and select the genotypes. In addition, environmental variation coefficient values below 4% indicated good experimental precision (Table 2).

Table 2. Analysis of variance of the number of whorl and number of basal roots for 1,000 common bean accessions.

Source of variation	GL	Number of whorls			Number of basal roots		
		SQ	QM	F	SQ	QM	F
Genotypes	999	223.90	0.22	147.45**	3758.24	3.76	38.86**
Residue	2000	3.04	0.001		193.63	0.1	
Total	2999	226.94			3951.87		
CV _e (%)		1.88			3.85		
CV _G (%)		13.12			13.66		
Genotypic Coefficient of Determination (%)		99.32			97.43		

**Significant by the F test at P <1%.

The number of whorls varied between 1 and 4 among genotypes, with an overall average of 2.07 for the 1000 accessions. The number of basal roots varied between 3 and 16, with an overall average of 8.09. There was a positive correlation between the number of whorls and basal roots with genotypes having the highest number of whorls also having the highest number of basal roots. A total of 55 and 64 genotypes had averages greater than or equal to 3 whorls and 10 basal roots, respectively. Vieira et al. (2008) reported that the number of whorls varied from 1.47 to 3.07, and the number of basal roots ranged from 5.67 (genotype TO) to 12.07 (cultivar Jalo MG-65) for 19 common beans genotypes. The Mahalanobis distance matrix and the Tocher cluster analysis (optimization) revealed seven different clusters for these two traits (Figure 1).

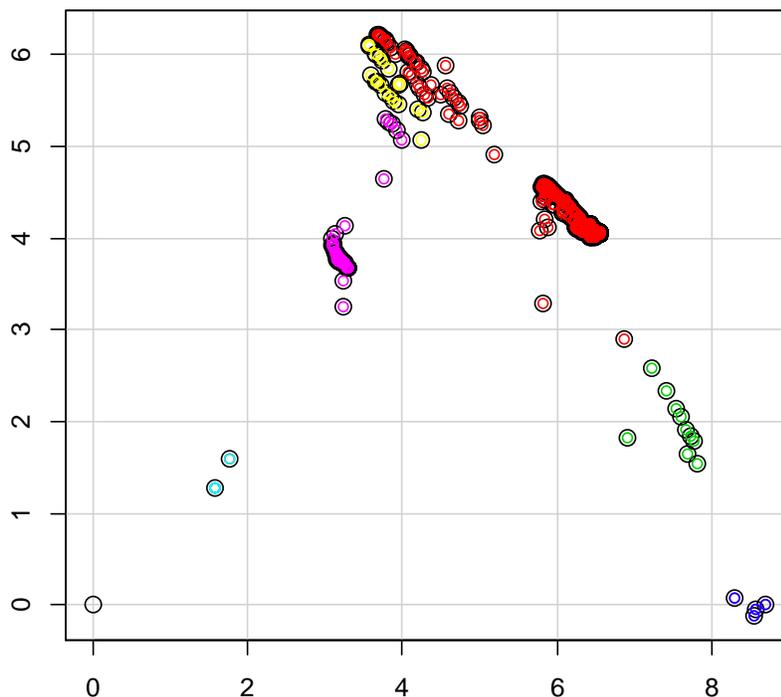


Figure 1. Biplot of the Mahalanobis generalized distance for number of root whorls and basal roots for the 1000 common bean accessions clustered by the Tocher algorithm.

Cluster 1, the largest, had 904 accessions, corresponding to 90.4% of the accessions and indicating that most of the accessions had similar numbers of whorls (mean of 2.01 whorls per seedling), and basal roots (mean of 7.95 basal roots per seedling). The other clusters included: Cluster 2 with 59 accessions (5.9%); Cluster 3, 10 (1%); Cluster 4, 4 (0.4%); Cluster 5, 20 (2%); Cluster 6, 2 (0.2%); and, Cluster 7, 1. Of the total accessions, the genotype 457 (Cavalo amarelo) was not clustered and stood out due to the highest number of whorls (average of 4 whorls per seedling) and basal roots (average of 14.86 basal roots per seedling).

The smallest and greatest distances observed were between Clusters 6 and 7 (131.556), and Clusters 4 and 7 (5921.835), respectively. The greatest intragroup distance was for Cluster 2 (24.044) with 59 accessions, and the shortest intragroup distance Cluster 4 (1.707) with four accessions.

The clustering method allows determination of which parents would be useful for obtaining new hybrids, based on the magnitude of their dissimilarity and the potential of the progenitors. Accessions grouped in the most distal clusters are the most genetically dissimilar and thus adequate for crossings. It is well known that individuals with the same dissimilarity pattern should not be crossed to avoid restricting genetic variability and prevent negative effects on selection gains. However, in this case, it is necessary to check whether the numbers of whorls and basal roots are correlated with traits of interest, such as grain yield. Based on Tocher divergent clusters, 47 genotypes were selected for the number of whorls and basal roots. These represented the genetic dissimilarity among all accessions for water deficit assessment at the pre-flowering stage (Table 3).

Table 3. Number of clusters formed by the Tocher optimization method (NCT), number of genotypes per cluster (NGC), number of genotypes selected per cluster (NGSC), average number of whorls (NW) and average number of basal roots (NBR).

NCT	NGC	NGSC	Selected Genotypes								NW	NBR
1	904	24	33	221	246	288	325	481	501	514	2.01	7.95
			575	593	633	672	707	712	742	832		
			844	1139	1183	1703	1979	1996	2118	2132		
2	59	7	84	499	561	613	699	1180	1181	2.94	11.35	
3	10	4	40	203	216	474				1.98	5.78	
4	4	3	89	95	485					1	4.24	
5	20	6	101	531	729	2000	2096	2143		2.48	9.68	
6	2	2	592	1698						3.56	14.06	
7	1	1	457							4	14.86	

According to Fageria et al. (1991), water stress conditioning during pre-blooming is the most effective, since this period is the most physiologically critical, directly affecting pod formation and yield. To examine the effectiveness of the water deficit, the plants' stomatal conductance was evaluated after stress induction. A highly significant effect ($P < 0.01$) of water treatment was observed, confirming that the water deficit was indeed effective. Also, the high coefficient of variation obtained for the plots reflects the amplitude values of the different genotypes analyzed. No significant differences were observed among genotypes for this trait, meaning that genotypes reduced their stomatal conductance rates as a defense mechanism against drought. Moreover, a significant effect was observed for the genotype x water treatment interaction, demonstrating the influence of the treatments on each genotype's performance (Table 4).

Table 4. Summary of variance analysis for stomatal conductance (SC), root length (RL), root dry mass (RDM), root surface area (RSA), root volume (RV), average root diameter (RD), number of seeds per plant (NSP), number of pods per plant (NPP) and grain yield (Y) of 47 common bean accessions.

VS	DF	Mean Square								
		SC ^t	RL ^t	MSR ^t	RSA ^t	RV ^t	RD ^t	NSP ^t	NPP ^t	Y ^t
Water										
Treatment (WT)	1	7857.75**	3823.85*	0.15	389.35*	1.89*	0.01	118.35**	5.32**	31.82**
Error aE	4	258.97	387.00	0.03	31.06	0.14	0.01	0.66	0.03	0.30
(G) Genotype	46	27.61	315.87**	0.02**	29.81**	0.16**	0.01**	42100.00**	0.19**	0.44**
WT X G	46	32.49 *	146.54	0.01	12.59	0.06	0.01	14.65	0.06	0.28
Error b	184	22.02	161.79	0.01	14.09	0.07	0.01	10.93	0.05	0.26
Overall	281									
CV (a)	%	108.27	44.63	14.16	39.59	22.56	2.83	17.83	7.07	21.90
CV (b)	%	31.57	28.85	7.18	26.67	16.19	1.67	22.88	9.37	20.39

*,** Significant at 5 and 1%, respectively, by *F test*. ^tTransformed data by $\sqrt{x+1}$

The water deficit during pre-blooming reduced stomatal conductance in most accessions; with plants undergoing drought treatment averaging $106.701 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ while those undergoing irrigation treatment averaging $444.511 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, with a 76% difference between the two. Table 5 shows stomatal conductance of drought treatment plants, which varied from the highest, $261.33 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ for access 101 (Plant \neq Type 1), to the lowest, $37.40 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ for access 1979 (ALB 213).

According to O'Toole et al. (1977) stomatal control is one of the main adaptation mechanisms of beans to drought since plants limit water loss by partially closing the stomata, consequently reducing gas exchange as a response to soil water restrictions. Therefore, the lowest values observed for the drought treatment characterize a defense mechanism of the plants, in which the stomata close partially to avoid water loss by the foliage during critical water deficit periods, as reported by Oliveira et al. (2005) for bean crops under different water conditions.

Table 4 shows the analysis of variance for the root system components. The water treatments affected the root length, root surface area, and root volume traits significantly, demonstrating that drought reduced root development. However, the root diameter trait, with a lower environmental coefficient, was not affected. It was also possible to observe significant effect of genotype on all traits, confirming the existence of genetic variability among accessions and non-significance for the interaction drought treatment x genotype for all these traits.

Root length varied between 943 and 4380 cm (Table 5), showing high variability among genotypes. Accessions 575 (RAI 76), 699 (Bayo), 514 (CF 830,186 Santa Rosa), 613 (D. Calima) and 95 (2227 Mulato branco) had the highest values, while genotypes 101 (Plant Type \neq 1), 672 (Iapar 81), 1996 (BFS 39), 1703 (IAC Jabola) and 474 (73 Vul 6686) had the lowest.

Bouma et al. (2000) stated that root length determines a plant's ability to absorb water and nutrients. He also stated that root systems of the same total length, but with different architectures, have a wide variation in root volume and interaction with nutrients. Hence, they emphasized that genotypes with similar root length may have different nutrient absorption rates and thus different productivities.

Table 5. Average stomatal conductance (SC), root length (RL), root dry mass (RDM), root surface area (RSA), root volume (RV) and average root diameter (RD), number of seeds per plant (NSP), number of pods per plant (NPP) and grain yield (Y) of 47 common bean accessions subject to the Irrigated (IR) and under water deficit (WD) treatments. The SC trait was investigated due to the interaction water treatment x accessions shown by the variance analysis, while the other traits averages were evaluated in a joint factorial design.

Accessions	SC IR	SC WD	RL	RSA	RV	RD	RDM	NSP	NPP	Y
33	324 A	173 A	1604 b	155 b	1.20 c	0.31 b	0.21 b	11 b	3 b	894 a
40	525 A	122 B	1839 b	171 b	1.28 c	0.29 b	0.18 b	15 b	4 b	640 a
84	386 A	74 B	1485 b	154 b	1.30 c	0.33 b	0.26 b	28 a	6 b	1320 a
89	341 A	72 B	2843 a	292 a	2.40 b	0.33 b	0.42 a	32 a	8 a	1248 a
95	453 A	127 B	3400 a	318 a	2.38 b	0.30 b	0.47 a	28 a	8 a	1300 a
101	333 A	261 A	943 b	90 b	0.70 c	0.31 b	0.10 b	8 b	2 b	470 a
203	463 A	132 B	2136 b	224 b	1.91 b	0.34 a	0.33 b	22 b	5 b	1016 a
216	322 A	51 B	2780 a	264 a	2.04 b	0.30 b	0.42 a	38 a	9 a	1564 a
221	255 A	188 A	2279 b	237 a	1.98 b	0.34 a	0.39 a	35 a	9 a	1284 a
246	436 A	82 B	2691 a	304 a	2.75 a	0.36 a	0.52 a	27 a	6 a	1350 a
288	399 A	75 B	1776 b	178 b	1.42 c	0.32 b	0.23 b	21 b	5 b	990 a
325	640 A	119 B	1605 b	148 b	1.10 c	0.29 b	0.32 b	20 b	5 b	950 a
457	595 A	46 B	1964 b	199 b	1.64 c	0.33 b	0.32 b	10 b	3 b	822 a
474	242 A	201 A	1427 b	181 b	1.86 b	0.41 a	0.36 a	31 a	7 a	1490 a
481	225 A	111 A	2836 a	255 a	1.85 b	0.31 b	0.37 a	27 a	6 a	1186 a
485	463 A	148 B	1675 b	166 b	1.36 c	0.31 b	0.23 b	13 b	6 a	810 a
499	617 A	159 B	1663 b	170 b	1.39 c	0.32 b	0.26 b	20 b	4 b	1436 a
501	577 A	39 B	2197 b	218 b	1.75 c	0.33 b	0.33 b	27 a	6 a	1160 a
514	379 A	113 B	3458 a	297 a	2.05 b	0.29 b	0.45 a	26 a	6 a	1456 a
531	617 A	121 B	2579 a	280 a	2.31 b	0.34 a	0.51 a	41 a	10 a	1692 a
561	340 A	81 B	1532 b	151 b	1.19 c	0.31 b	0.22 b	12 b	3 b	1026 a
575	495 A	61 B	4380 a	440 a	3.57 a	0.32 b	0.71 a	33 a	7 a	1262 a
592	430 A	120 B	1842 b	193 b	1.66 c	0.34 a	0.36 a	13 b	5 b	1190 a
593	652 A	101 B	1960 b	198 b	1.60 c	0.30 b	0.35 a	31 a	6 a	1320 a
613	265 A	88 A	3415 a	344 a	2.77 a	0.32 b	0.44 a	10 b	2 b	1032 a
633	278 A	73 A	1748 b	175 b	1.43 c	0.31 b	0.25 b	21 b	5 b	848 a
672	321 A	171 A	989 b	112 b	1.03 c	0.39 a	0.18 b	19 b	5 b	954 a
699	374 A	38 B	3629 a	390 a	3.40 a	0.36 a	0.63 a	12 b	4 b	1420 a
707	719 A	111 B	2212 b	228 b	1.91 b	0.34 a	0.37 a	23 a	5 b	1204 a
712	798 A	75 B	2243 b	243 a	2.12 b	0.35 a	0.53 a	36 a	8 a	1914 a
729	396 A	110 B	2701 a	249 a	1.88 b	0.30 b	0.41 a	24 a	6 b	1142 a
742	277 A	52 B	2324 b	200 b	1.42 c	0.28 b	0.32 b	14 b	3 b	778 a
832	191 A	84 A	1566 b	183 b	1.74 c	0.35 a	0.36 a	26 a	5 b	1434 a
844	515 A	106 B	2714 a	256 a	2.40 b	0.33 b	0.45 a	13 b	3 b	952 a
1139	448 A	133 B	1640 b	153 b	1.17 c	0.32 b	0.24 b	21 b	4 b	1172 a
1180	783 A	119 B	1590 b	163 b	1.36 c	0.35 a	0.27 b	17 b	4 b	1044 a
1181	390 A	92 B	1567 b	169 b	1.46 c	0.36 a	0.26 b	20 b	5 b	1038 a
1183	482 A	91 B	2601 a	257 a	2.04 b	0.31 b	0.40 a	21 b	6 b	1078 a
1698	458 A	139 B	2298 b	214 b	1.50 c	0.32 b	0.34 b	13 b	3 b	926 a
1703	598 A	128 B	1348 b	141 b	1.22 c	0.36 a	0.21 b	13 b	3 b	1186 a
1979	304 A	37 B	2736 a	266 a	2.08 b	0.31 b	0.39 a	18 b	4 b	1252 a
1996	555 A	127 B	1245 b	138 b	1.23 c	0.35 a	0.23 b	24 a	5 b	1410 a
2000	857 A	75 B	2107 b	207 b	1.62 c	0.31 b	0.29 b	18 b	5 b	1158 a
2096	321 A	62 B	1550 b	157 b	1.27 c	0.32 b	0.21 b	13 b	3 b	760 a
2118	165 A	161 A	2235 b	238 a	2.05 b	0.33 b	0.39 a	27 a	7 a	1438 a
2132	470 A	95 B	1663 b	161 b	1.26 c	0.31 b	0.26 b	20 b	6 b	1150 a
2143	418 A	75 B	1634 b	155 b	1.18 c	0.31 b	0.24 b	17 b	4 b	902 a

* Average values followed by lowercase letters differ from each other for genotypes and average values with different capital letters differ from each other for water treatments by the Scott-Knott test ($P < 0.05$).

The root surface area varied between 90 and 440 cm², showing high variability among genotypes. Accessions 575 (RAI 76), 699 (Bayo), 613 (D. Calima), 95 (2227 Mulato branco) and 246 (56 Retinto Santa Rosa) displayed the highest average while accessions 101 (Plant Type ≠ 1), 672 (Iapar 81), 1996 (BFS 39), 1703 (IAC Jabola) and 325 (Rosinha G2) had the worst performance (Table 6). The root volume varied from 0.70 to 3.57 cm³. The highest root volumes were observed for accessions 575 (RAI 76), 699 (Bayo), 613 (D. Calima), 246 (56 Retinto Santa Rosa) and 844 (INB 816) while the worst were 101 (Plant Type ≠ 1), 672 (Iapar 81), 325 (Rosinha G2), 1139 (IAC Rubi) and 2143 (SMC 9) (Table 5).

Table 6. Pearson correlation for stomatal conductance (SC), root length (RL), root surface area (RSA), root volume (RV), root average diameter (RD), root dry mass (RDM), number of seeds per plant (NSP), number of pods per plant (NPP), grain yield (Y), number of whorls (NW), and number of basal roots (NBR) under drought.

	RL	RSA	RV	RD	RDM	NSP	NPP	Y	NW	NBR
SC	-0.2895 *	-0.2669	-0.2332	0.0955	-0.2395	-0.0688	-0.0619	-0.1034	-0.1266	-0.1110
RL		0.9392 **	0.8286 **	-0.2901 *	0.7650 **	0.2680	0.1649	0.2327	-0.1268	-0.1285
RSA			0.9501 **	-0.0143	0.8595 **	0.3296 *	0.2658	0.3347 *	-0.1198	-0.1240
RV				0.2295	0.8825 **	0.3192 *	0.3032 *	0.3663 *	-0.1338	-0.1398
RD					0.1493	0.0529	0.1450	0.2011	0.0631	0.0629
RDM						0.3926 **	0.3171 *	0.4782 **	-0.0376	-0.0311
NSP							0.8275 **	0.8358 **	-0.3449 *	-0.3642 *
NPP								0.7051 **	-0.4270 **	-0.4327 **
Y									-0.1449	-0.1327
NW										0.9790 **

** , * : Pearson test significant at 1% and 5%, respectively.

The average root diameter ranged from 0.41 to 0.28 mm. The accessions with the largest diameter were 474 (6686 Vul 73), 672 (Iapar 81), 1703 (IAC Jabola), 699 (Bayo), and 1181 (IAC Galante) while the smallest were for 742 (S. 309-1), 514 (CF 810510 Santa Rosa), 325 (Rosinha G2), 40 (Retinto Santa Rosa), and 95 (2227 Mulato branco) (Table 5).

Even though there was a significant effect by genotype on dry root mass, varying from 0.10 to 0.71 g, no such significance was apparent between the two water treatments, 0.28g for drought and 0.40 g, for irrigated. The accessions with the highest RDM were 575 (RAI 76), 699 (Bayo), 712 (IAC Bico de Ouro), 246 (56 Retinto Santa Rosa), and 531 (PI 310724), and the lowest, 101 (Plant Type \neq 1), 672 (Iapar 81), 40 (Retinto Santa Rosa), 1703 (IAC Jabola), and 2096 (SCR 17) (Table 5).

Analysis of variance (Table 4) showed that water treatments and genotypes affected all production traits significantly, thus demonstrating that water deficit directly affects the productivity of all genotypes. Drought reduced NSP, NVP, and PG by 56, 64 and 52%, respectively. As there was no significant difference between water treatment x genotype interaction, the best genotypes can be selected based on the joint analysis, although for some genotypes, the yield decreased less during the drought period.

The NSP varied between 8 and 41 seeds per plant (Table 5). The best performances were observed for 531 (PI 310724), 216 (91/71-212), 712 (IAC Bico de Ouro), 221 (12-D), and 575 (RAI 76) while the worst were 101 (Plant Type \neq 1), 613 (D. Calima), 457 (Cavalo Amarelo), 33 (Jalo), and 699 (Bayo). The NPP ranged between 2 and 10 pods per plant. The best performances were observed for 531 (PI 310724), 221 (12-D), 216 (91/71-212), 95 (2227 Mulato Branco) and 89 (Venezuela 350) and the worst results were observed for 613 (D. Calima), 101 (Plant Type \neq 1), 561 (Lagartixa Precoce), 457 (Cavalo Amarelo), and 1703 (IAC Jabola) (Table 5).

Drought treatment decreased the average grain yield to 793.6 kg.ha⁻¹ compared to 1504.2 kg.ha⁻¹ under irrigation. Thus, the water deficit intensity index was 52%, characterized as high, confirming the effectiveness of the treatment.

The grain yield varied between 470 and 1914 kg ha⁻¹, taking the averages of the two treatments. The accessions that performed best were 712 (IAC Bico de Ouro), 531 (PI310724), 216 (91/71-212), 474 (73 Vul 6686) and 514 (CF 820510 Santa

Rosa). The worst performances were observed for 101 (Plant Type \neq 1), 40 (Retinto Santa Rosa), 2096 (SCR 17), 742 (L. 309-1), and 485 (Dom Timoteo). The genotypes that presented good yields under drought conditions were 712 (IAC Bico de Ouro) and 2118 (SER 28), both classified in Cluster 1 by the Tocher algorithm. The productivity of IAC Bico de Ouro decreased only 2.3% (from 1936.6 kg.ha⁻¹ in the irrigated treatment to 1893.8 kg ha⁻¹ in the drought treatment). The productivity of genotype SER 28 (red integument) drought tolerant from CIAT decreased 12.5% (from 1533.6 kg ha⁻¹ in the irrigated treatment to 1342.4 kg ha⁻¹ in the drought treatment).

Porch et al. (2009) stated that the lines carrying the SEC, SEN, and SER acronyms have been recently released as drought tolerant in the small market classes of the cream, black, and red integument. In this context, the genotype 712 (IAC Bico de Ouro) with a cream integument stands out. This genotype performed better than the drought tolerant genotypes SER 28 and SER 210. Therefore, it could be used as parent in artificial crosses to develop new cultivars with the carioca integument, targeting the Brazilian market.

Genotypes 221 (12-D) with a black integument and 1181 (IAC Galante) with a rose integument were not the most productive under the drought treatment; however, they were considered drought tolerant as their grain yield reduction was low, 1.5 and 3.1%, respectively. As such, they could be important parents for new combinations, though preferably crossed with more productive accessions.

There was significant positive correlation between the root traits (dry mass, surface area, and volume) and grain yield, i.e., accessions that had greater development of the root system under water deficit, also showed higher numbers of pods and seeds, and higher grain yield (Table 6, Pearson correlation analysis). The traits number of whorls and number of basal roots had a significant negative correlation with the number of seeds and number of pods per plant; that is, larger numbers of whorls and basal roots resulted in smaller numbers of pods and seeds. There was no correlation between NW and NBR with grain yield, which can be, in part, explained by the malformation of pods and seeds under drought (Table 6).

Water deficit is an important cause of low productivity of bean crops. Therefore, we sought to evaluate the traits of the root system to find divergence among the investigated genotypes and determine whether the dissimilarity of the genotypes of each group resulted in different productive performances of crops submitted to water deficit. Thus, contrary what we observed, a positive correlation was expected between the traits initially measured (NW and NBR) with the production components and yield. However, the largest number of whorls and basal roots did not correspond to the most productive potential of genotypes under water deficit.

However, there was a positive and highly significant correlation ($P < 0.01$) between the root dry mass and grain yield, enabling the selection of genotypes that showed greater root development and higher production performance (Figure 2). Therefore, the most promising genotypes under water deficit conditions were: 22 (RAI 76) 10 (56 Retinto Santa Rosa), 45 (SER 28), 28 (Bayo), 29 (IAC Una), 30 (IAC Bico de Ouro), 9 (12d), 4 (Venezuela 350), 20 (PI 310724), 19 (CF 820510 Santa Rosa), 24 (EMP 407), 39 (IAC Boreal), 31 (L.476-4), and 41 (ALB 213).

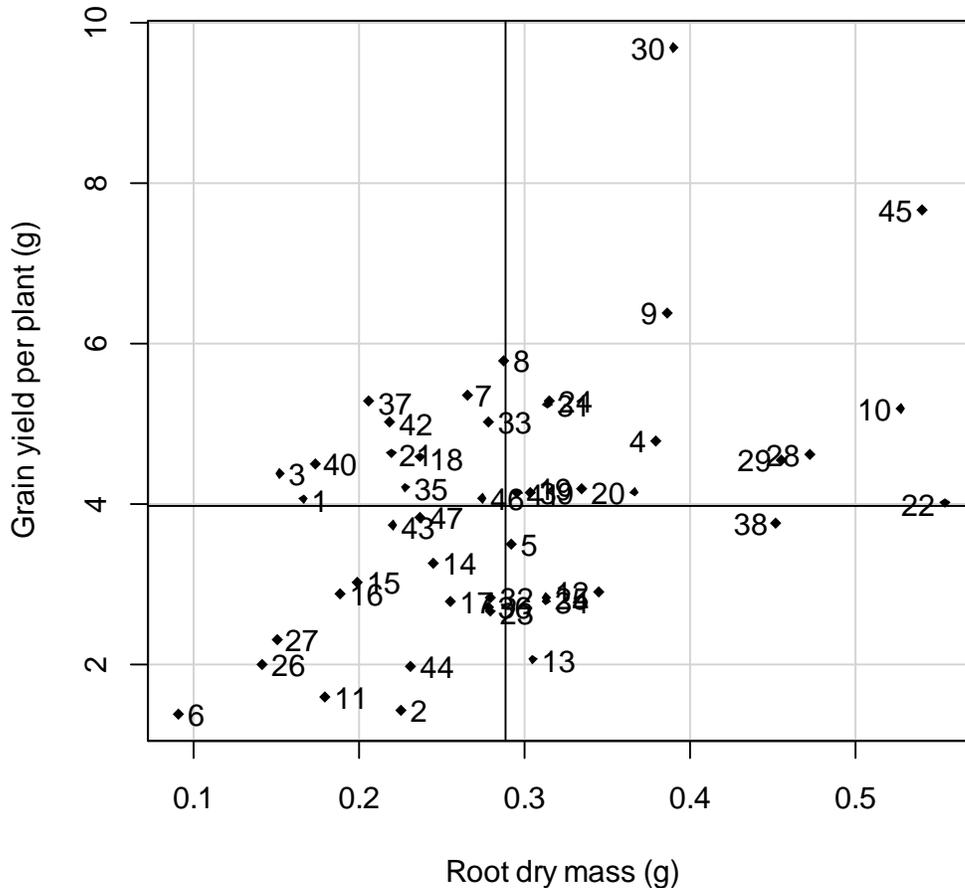


Figure 2. Performance of 47 bean genotypes for root dry mass production (g) (x-axis) and grain yield per plant (g) (y-axis) under water deficit. The horizontal and vertical lines drawn on the chart represent the overall genotypes averages.

CONCLUSIONS

The Common bean genotypes from the active genebank of the IAC showed wide diversity in the traits number of whorls and number of basal roots, leading to dissimilarity clusters under the Mahalanobis genetic divergence analysis and the Tocher optimization method. The applied water treatments were effective in distinguishing genotypes; significant differences could be observed in the development of plants and evaluated traits. The root system traits such as root dry mass, root surface area and root volume showed positive and significant correlations with grain yield under drought. The most promising genotypes for root growth and grain yield under water deficit were: RAI 76, 56 Retinto Santa Rosa, SER 28, Bayo, IAC Una, IAC Bico de Ouro, 9 12-D, Venezuela 350, PI 310724, CF 820510 Santa Rosa, EMP 407, IAC Boreal, L.476-4 and ALB 213. Those genotypes may be included in crossing blocks for obtaining new drought tolerant lines.

ACKNOWLEDGMENTS

FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo.
CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

REFERENCES

- Beebe SE, Rao IM, Cajiao C, Grajales M. (2008). Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. *Crop. Sci.* 48: 582-592.
- Blum A. (1997). Crop responses to drought and the interpretation of adaptation. In: BELHASSEN, E. Drought tolerance in higher plants: genetical, physiological, and molecular biology analysis. Dordrecht Kluwer Academic. p.57-70.
- Bouma TJ, Nielson KL, Koutsaal B. (2000). Sample preparations and scanning protocol for computerized analyses of root length and diameter. *Plant. Soil.* 218: 185-196.
- Ceolin AGC, Gonçalves-Vidigal MC, Vidigal-Filho PS, Kvitschal MV, Gonela A, Scapim CA. (2007). Genetic divergence of the common bean (*Phaseolus vulgaris* L.) group carioc using morpho-agronomic traits by multivariate analysis. *Hereditas* 141: 1-9.
- Chiorato AF, Carbonell SAM, Colombo CA, Dias LAS. (2005). Genetic diversity of common bean accessions in the germoplasm bank of the Instituto Agrônômico – IAC. *Crop. Breed. Appl. Biotechnol.* 5: 1-9.
- CIAT (Centro Internacional de Agricultura Tropical) (2012). Phenotyping common beans for adaptation to drought: protocol for field evaluation. Available at: <<http://ciat.cgiar.org/beans/default.aspx>>. Accessed: 15 December 2018.
- Cornic G, Briantais JM. (1990). Partitioning of photosynthetic electron flow between CO₂ and O₂ reduction in a C₃ leaf (*Phaseolus vulgaris* L.) at different CO₂ concentrations and during drought stress. *Planta.* 183: 178-184.
- Cruz, CD. (2006). Programa Genes: Biometria. Genes Program: Biometrics. Editora UFV.
- Fageria NK, Baligar VC, Jones CA. (1991). Common Bean And Cowpea. In: Fageria, N. K.; Baligar, V. C.; Jones, C. A. Growth and mineral nutrition of field crops. New York: Maecel Dekker. p. 280-318.
- Liao H, Rubio G, Yan X, Cao A, Brown KM, Lynch JP. (2001). Effect of phosphorus availability on basal roots shallowness in common bean. *Plant. Soil.* 232: 69-79.
- McClellan PE, Burridge J, Beebe S, Rao IM, Porch TG. (2011). Crop improvement in the era of climate change: an integrated, multi-disciplinary approach for common bean (*Phaseolus vulgaris*). *Funct. Plant Biol.* 38: 927-933.
- Mahalanobis PC. (1936). On the generalized distance in statistics. *Proc. Nat. Inst. Sci. India.* 1: 49-55.
- Molina JC, Moda-Cirino V, Fonseca-Júnior NSF, Faria RT, Destro D. (2001). Response of common bean cultivars and lines to water stress. *Crop Breed. Appl. Biotechnol.* 1: 363-372.
- O' Toole JC, Ozburn JL, Wallace DH. (1977). Photosynthetic response to water stress in *Phaseolus vulgaris* L. *Physiol. Plant.* 40: 111-114.
- Oliveira AD, Fernandes EJ, Rodrigues TJD. (2005). Condutância estomática como indicador de estresse hídrico em feijão. *Eng. Agric.* 25: 86-95.
- Pimentel C, Hébert G, Silva JV. (1999a). Effectes of drought on O₂ evolution and stomatal conductance of beans at the pollination stage. *Environ. Exp. Bot.* 42: 155-162.
- Pimentel C, Laffray D, Louguet P. (1999 b). Intrinsic Water Use Efficiency at the Pollination Stage as a Parameter for Drought Tolerance Selection in *Phaseolus vulgaris*. *Physiol. Plant.* 106: 184-189.
- Polanía JA, Rao IM, Beebe S, García R. (2009). Desarrollo y distribución de raíces bajo estrés por sequía en frijol común (*Phaseolus vulgaris* L.) en un sistema de tubos con suelo. *Agron. Colomb.* 27: 25-32.
- Porch TG, Ramirez VH, Santana D, Harmsen EW. (2009). Evaluation of common bean for drought tolerance in Juana Diaz, Puerto Rico. *J. Agron. y Crop. Sci.* 195: 328-334.
- R Development Core Team (2012). R: A language and environment for statistical computing. Foundation for Statistical Computing, Austria. ISBN 3-900051-07-0. URL <http://www.R-project.org>.
- Rubio G, Lynch JP (2007). Compensation among root classes in *Phaseolus vulgaris* L. *Plant. Soil.* 290: 307-321.
- Santos MG, Ribeiro RV, Oliveira RF, Pimentel C. (2004). Gas exchange and yield response to foliar phosphorus application in *Phaseolus vulgaris* L. under drought. *Brazi. J. Plant Physiol.* 16: 171-179.
- Santos MG, Ribeiro RV, Teixeira MG, Oliveira RF, Pimentel C. (2006a). Foliar phosphorus supply and CO₂ assimilation in common bean (*Phaseolus vulgaris* L.) under water deficit. *Brazi. J. Plant Physiol.* 18: 407-411.
- Santos MG, Ribeiro RV, Oliveira RF, Machado EC, Pimentel C. (2006b). The role of inorganic phosphate on photosynthesis recovery of common bean after a mild water deficit. *Plant Sci.* 170: 659-664.
- Sawasaki HE, Teixeira JPF. (1981). Estresse de água no crescimento, produtividade e acúmulo de prolina em feijão. *Bragantia* 4: 157-166.
- Singh SP. (2007). Drought resistance in the race durango dry bean landraces and cultivars. *Agron. J.* 99: 1219-1225.

- Terán H, Singh SP. (2002). Comparison of sources and lines selected for drought resistance in common bean. *Crop. Sci.* 42: 62-70.
- Upadhyaya HD, Gowda CLL, Sastry DVSSR. (2008). Plant genetic resources management: collection, characterization, conservation and utilization. *J. SAT. Agricult. Res.* 6: 1-16.
- Vieira RF, Jochua CN, Lynch JP. (2007). Method for evaluation of roots hairs of common bean genotypes. *Pesq. Agropec. Bras.* 42: 1365-1368.
- Vieira RF, Carneiro JES, Lynch JP. (2008). Root traits of common bean genotypes used in breeding programs for disease resistance. *Pesq. Agropec. Bras.* 43: 707-712.