

QUANTITATIVE TRAIT LOCI IDENTIFIED FOR ROOT TRAITS ASSOCIATED WITH DROUGHT RESISTANCE IN COMMON BEAN

Boris Briñez¹, Isabella Laporte Santos¹, Caléo Panhoca de Almeida¹, João Guilherme Ribeiro Gonçalves², José Antônio de Fátima Esteves², Alisson Fernando Chioratto², Sérgio Augusto Morais Carbonell², Idupulapati Madhusudana Rao³, Luciana Lasry Benchimol-Reis¹

1) Instituto Agrônomo (IAC), Centro de Recursos Genéticos Vegetais, 13075-630, Campinas, SP, Brazil;

2) Instituto Agrônomo (IAC), Centro de Grãos e Fibras, 13075-630, Campinas, SP, Brazil;

3) International Center for Tropical Agriculture (CIAT) Cali, Colombia.

* Corresponding author: Luciana Lasry Benchimol-Reis. e-mail: llasry@iac.sp.gov.br

Abstract: Common bean has a high sensitivity to drought stress, particularly during reproductive development which reduces its yield. In this study we aimed to: (i) evaluate differences in shoot and root response of a mapping population of 107 recombinant inbred lines (RILs) of SEA 5 × AND 277 cross under drought stress, and (ii) identify the QTLs associated with improved plant performance under water stress treatment. Phenotyping was performed under greenhouse conditions where the plants were grown using rhizotrons under well-watered and water-stress conditions. Four QTLs were identified that are related to improved performance under water stress and three of them were related specifically to roots and these are located on chromosomes Pv1 and Pv6. Root superficial area trait explained 32.6% of the variance and may contribute to greater water uptake and improved adaptation of common bean under water stress conditions.

Keywords: *Phaseolus vulgaris* L., rhizotron, linkage mapping, water stress, molecular markers.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important staple foods in tropical Latin America, and Eastern Africa. It is also one of the most important diet constituents for many people across the globe for obtaining protein, carbohydrate, and iron (Campos-Veja et al., 2010).

The crop yield is reduced due to drought stress (both intermittent and terminal), which affect about 60% of the common bean cultivation area globally (Beebe et al., 2013). In Latin America, the water requirements of the crop cycle are not satisfied in 93% of the bean cultivation areas (Singh, 1995), and a huge fraction of the yield reduction can be avoided through breeding and harvesting of drought-tolerant cultivars (Subbarao et al., 1995). A considerable number of physiological, morphological, and phenological factors are involved in improving adaptation to drought (Beebe et al., 2013; Rao et al., 2013; Rao, 2014; Polania et al., 2017a, b; Lanna et al., 2018).

The Durango race has been reported to possess a superior performance under drought and is suited for developing new varieties tolerant to water deficit conditions (Singh et al., 2001; Singh, 2007). Crossing between Durango and Mesoamerica races (Singh et al., 1991) is an approach used for achieving a superior response to drought stress (Mukeshimana et al., 2014). The CIAT (International Center for Tropical Agriculture) bred line SEA 5 is well-adapted to drought stress (Singh et al., 2001; Polania et al., 2017a). It is derived from inter-racial crosses between the Mesoamerica and Durango races, it has small (22–25g 100 seed⁻¹) cream-colored seeds and Type III growth habit. It is also resistant to *Fusarium* root rot and has the I gene for resistance to bean common mosaic virus (BCMV). Furthermore, it has greater ability for photosynthate remobilization (Mukeshimana et al., 2014; Polania et al., 2017a). On the other hand, the Andean cultivar AND 277 belongs to the Nueva Granada race (Blair et al., 2009), derived from the crosses between [Cargabello x (Pompadour Checa x Línea 17) x (Línea 17 x Red Kloud)] and has cream-colored seed with red mottles. It is commonly used in breeding programs as a source of resistance because the *Co-1⁴* and the *Phg-1* alleles that confer resistance to the anthracnose and angular leaf spot

pathogens, respectively (Gonçalves-Vidigal et al., 2011).

Common bean's diversity in root architecture contributes to improved water extraction from deeper soil layers during drought stress (Lynch, 2018; Strock et al., 2019). Previous research reported antecedence and profound rooting for drought avoidance (Beebe et al., 2013) and a superior photosynthate remobilization (Polania et al., 2016; Rao et al., 2017) directly contributing to improved grain filling (Lynch, 1995; Rao et al., 2017; Polania et al., 2017b). Furthermore, Polania et al. (2017b) described two classes of ideotypes of water use; water savers and water spenders and their association with specific root traits under drought conditions. Water spenders were superior in their grain yield and this was related to a higher root vigor with deeper rooting ability.

In a previously study, Mukeshimana (2014) discovered 14 QTLs for improved performance under drought stress (DS) in different environments using 2,122 SNPs from BARCBean6k_3 Beadchip and yield QTLs occurred mainly on chromosomes Pv03 and Pv09. In addition, a single QTL related to yield under DS on Pv09 was derived from SEA 5 parental line. Briñez et al. (2017) also identified 8 QTLs for drought resistance in vase experiment placed inside greenhouses (overground experiment). Most of the QTLs under water stress had the SEA 5 allele contribution.

The aim of this study was to evaluate differences in shoot and root response of two parents (SEA 5 and AND 277) and their 107 recombinant inbred lines under water stress and to identify the QTLs associated with improved root performance to contribute to breeding of common bean to drought-prone environments.

Material and methods

Plant materials

A set of 107 recombinant inbred lines (RILs) from the SEA 5 × AND 277 cross from CIAT (Cali, Colombia) was used. The population was advanced until the F8 generation, using the single seed descent (SSD).

SEA 5 was developed at CIAT (Singh et al., 2001) and it is superior to BAT 477 (Pérez Vega et

al., 2011) for yield under drought stress. AND 277 belongs to the Andean gene pool (Blair et al., 2009) and it is susceptible to water stress.

Phenotyping for drought resistance

The greenhouse experiment was carried out between March and September 2012 at the Agronomic Institute (IAC, Campinas, São Paulo, Brazil). It was performed in a completely randomized experimental design (CRD) with three replicates and with two contrasting water supply conditions, well-watered (WW) and the water stress (WS) treatments. Two hundred and fourteen rhizotrons (60 cm long, 24 cm diameter plastic cylinders) were filled with 0.013565 m³ of a soil: sand (2:1 w/w) mix in each cylinder. The soil-sand substrates were thoroughly mixed and fertilized with a rate of NPK 8-18-16.

The pre-germinated seeds were previously sterilized with 5% calcium hypochlorite solution for 5 min, dried at room temperature, and then sown in the soil. They were irrigated with 400 mL of water per day until the water stress treatment was applied. A total of 20 watermark sensors (granular matrix sensor, Irrrometer Company, www.irrometer.com, Riverside, CA) were randomly placed at 20 cm downward in the soil to monitor soil water in the WW and WS treatment rhizotrons. The soil water tension and the leaf temperature of the plants that had the watermark sensors under them were measured every two days.

The mean leaf temperature of the WS treatment was 22.9 °C while it was 22.6 °C for the WW treatment. The average ambient temperature of the greenhouse was 25.5 °C and the relative humidity was 33.8%. The plants in WW treatment were watered to 80% of the water holding capacity (ability of the soil to absorb water) and the plants in WS treatment were maintained in the process of soil drying to simulate terminal drought stress conditions. Soil drying for WS treatment was applied during the vegetative phase (V3/V4). The WS treatment received no water from day 25 after sowing. The plants were harvested at 52 days after sowing and the morpho-physiological characteristics were measured.

Leaf temperature was registered using an infrared thermometer (Telatemp model AG-42D, Telatemp, CA, USA). The chlorophyll levels in the leaves were assessed using a SPAD-502 meter (Konica Minolta Chlorophyll Meter SPAD-502 Plus, Osaka Japan). Leaf area was evaluated by

LICOR (model LI-3000). Stem and leaf fresh biomass were determined using an analytical balance (BEL engineering, Milan, Italy). Bean plant parts were dried in incubator at 60 °C, for four days, inside paper bags, and subsequently had their weights determined. Roots were individually collected and washed with clean water, followed by 5% soap, 1% soap, and finally, distilled clean water. After they had been washed, each plant root was placed in a separate container in 20% alcohol solution until analysis. The roots were scanned, and the images examined in detail with WinRHIZO software (Regent Instruments Inc., Quebec, Canada) to determine root length (cm plant⁻¹), root superficial area (cm² plant⁻¹), and root volume (cm³ plant⁻¹).

Phenotypic analysis

ANOVA test (analyses of variance) for all characteristics measured in both WW and WS treatments was performed using General Linear Models Procedure (GLM) and the SAS v.8.2 program (SAS Institute, Cary, NC, USA). Parents and derived lines were individually analyzed so that the contrasting traits among the genotypes could be confirmed. The ANOVA was significant when $P < 0.05$. PCA (principal component analysis) was performed with PAST3 software (Hammer et al., 2001).

Molecular markers and QTL mapping

Two different classes of co-dominant molecular markers were used in this study: microsatellites (SSRs) and Single Nucleotide Sequences (SNPs) such as described in (Briñez et al., 2017). Extraction of DNA was performed from 300 mg of powdered lyophilized leaves with CTAB protocol (CIMMYT, 2005). It was quantified and diluted to 100 ng μL^{-1} . The polymorphism was evaluated among the parents using 594 SSRs and 384 SNPs (Müller et al., 2015). The OneMap software version 2.1.2 (Margarido et al., 2007) was used for genetic mapping by multipoint approaches and hidden Markov models.

Results and discussion

Roots are connected to the rest of the plant through signaling pathways (Paez-Garcia et al., 2015). There is a distinct diversity in root system development under drought conditions (Polania et al. 2017b; Strock et al., 2019) and drought resistance

is positively related with a vigorous and deeper root system (Polania et al., 2017a).

Finding associations between roots and genetic markers through QTL mapping may enhance the knowledge of how root architecture influences the physiological responses involved in drought resistance (Asfaw and Blair, 2012). Since this study used a cross between the drought resistant parent SEA 5 and drought sensitive parent AND 277 to generate recombinant inbred lines, this population was very suitable to identify QTLs related to improved performance under drought stress conditions.

ANOVA applied to 11 traits measured showed that there was significant variation between treatments, parents, and RILs (Table 1). Among SEA 5 and AND277 (parents), there were differences in leaf area (cm² plant⁻¹), leaf biomass (fresh, g plant⁻¹), stem biomass (dry, g plant⁻¹), leaf temperature (°C), root length (cm plant⁻¹), root superficial area (cm² plant⁻¹), and root volume (cm³ plant⁻¹). In contrast, the only trait in the WW treatment that was significantly different between the parents was leaf biomass (dry, g plant⁻¹). Leaf area was also identified as contrasting for parents in Boris et al. (2017); however, it was only evaluated

and mapped in this study (Table 2) and it explained 20.3% (R²) of the phenotypical variance of the character.

A biplot representation of a principal component analysis (PCA, Figure 1) identified that there was enough segregation and a distinctive behavior presented by the RILs. PC1 accounted for 94.95% of the variation in WS treatment. The second principal component accounted for 3.24% of the total variation in the data. Mainly, diversity between AND277 and SEA5 parents, which are nearest to PC1 axis, was mostly influenced by root length (RL). Some RILs, such as number 38, 21, 27, are mostly influenced by superficial root area (SRA) which was negatively correlated to LA trait.

Among the 594 microsatellite markers selected for the parents, 150 (25%) were polymorphic for the population and 80 SSRs (53.3%) were mapped. A total of 288 polymorphic markers were identified in SNPs and 251 were included in the genetic map. Some of the markers (9.37%) were still in heterozygote state and were not included in the analysis. The AS map was constructed with a total of 331 segregating markers and covered the 11 bean chromosomes with a total length of 1,515.2 cM.

Table 1. Analyses of variance comparison of the quantitative traits for AND 277, SEA 5, and the recombinant inbred lines of the AND-277×SEA5 population (*Phaseolus vulgaris* L.) that were evaluated under well-watered and water stress conditions using rhizotrons in a greenhouse.

Trait	Well-watered			RILs Average	h ² _g	Water stress			RILs Average	h ² _g
	Parents					Parents				
	SEA 5	AND 277	Diff			SEA 5	AND 277	Diff		
Chlorophyll	40.55	40.1	ns	39.84*	0.85	42.63	36.25	ns	38.17*	0.61
Leaf area (cm ² plant ⁻¹)	485	594.8	ns	391.99*	0.78	410.63	179.1	*	217.47*	0.3
Leaf biomass (Fresh g plant ⁻¹)	5.68	7.25	ns	4.23*	0.75	3.61	1.82	*	1.99 ^{ns}	0.05
Stem biomass (Fresh g plant ⁻¹)	4.55	4.48	ns	4.13 ^{ns}	0.17	2.31	3.23	ns	2.20 ^{ns}	0.01
Leaf biomass (dry, g plant ⁻¹)	0.81	1.9	*	0.93 ^{ns}	0.13	1.41	0.78	ns	0.66 ^{ns}	0.11
Stem biomass (dry, g plant ⁻¹)	0.41	0.98	ns	0.7 ^{ns}	0.06	0.73	1.59	*	0.53 ^{ns}	0.13
Leaf temperature (°C)	19.5	19	ns	19.08 ^{ns}	0.2	21.66	19	*	20.86*	0.4
Root length (cm plant ⁻¹)	1248	2222.5	ns	1847.81 ^{ns}	0.07	2371.4	1079.6	*	1775.72*	0.3
Root superficial area (cm ² plant ⁻¹)	135.62	205.33	ns	181.97*	0.45	239.63	93.89	*	198.54*	0.82
Root volume (cm ³ plant ⁻¹)	1.17	1.5	ns	1.49*	0.64	1.41	0.59	*	0.95*	0.21
Root diameter (mm)	0.35	0.29	ns	0.3 ^{ns}	0.01	0.26	0.25	ns	0.26 ^{ns}	0.07

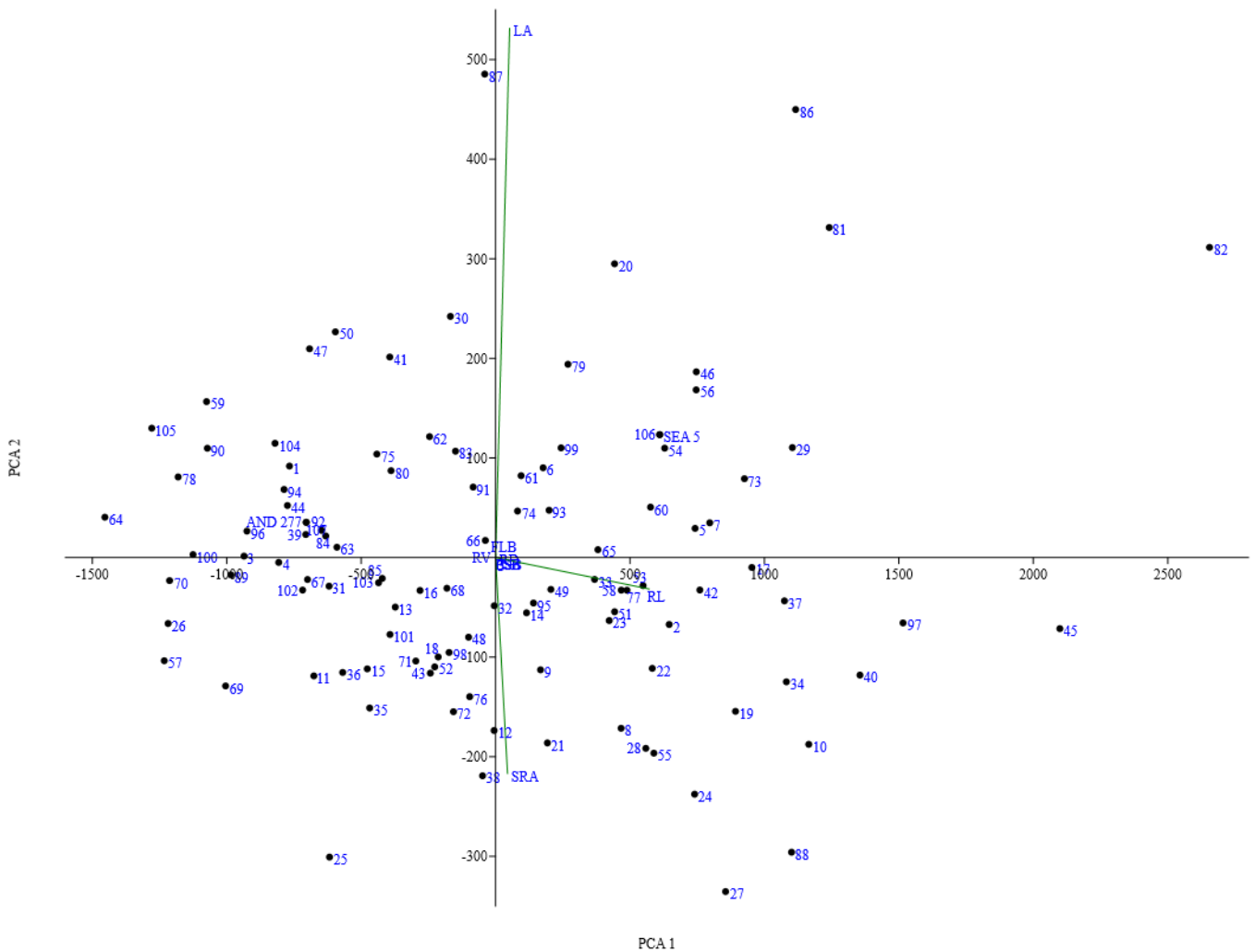


Figure 1. PCA Biplot of observations and variables obtained from leaf area (LA; $\text{cm}^2 \text{plant}^{-1}$), root length (RL, cm plant^{-1}) and superficial root area (SRA, $\text{cm}^2 \text{plant}^{-1}$) from AND-277 \times SEA5 population. Numbered black dots are the RILs. PC1 the first principal component explaining 94,95% of the variance and PC2: the second principal component explaining 3.24% of the variance.

Most QTLs (15) were identified with the WW treatment while four QTLs were identified with the WS treatment. These 4 QTLs were found in chromosome Pv1 and Pv6 for three traits (leaf area – 32.6%, root length – 20.3%, superficial root area – 19.20%, Table 2, Figure 2).

Most of the QTLs with the greatest effects for both treatments (WW and WS) had contribution from the SEA 5 allele. The QTLs LA1.1^{AS} (Table 2, WS), SBF1.1^{AS} (Table 3, WW), LBF1.1^{AS} (Table 3, WW), and RSA1.1^{AS} (Table 3, WW) were in the same marker interval and had the same nearest marker, which was PVBR03 on chromosome Pv1. The QTLs SBF3.3^{AS} and SBD3.1^{AS} (Table 3, WW) had marker BM189 on chromosome Pv3, and the QTLs RL6.1^{AS} and RSA6.1^{AS} showed marker BM 3 on chromosome Pv6. The QTLs were identified for all the traits analyzed, apart from leaf temperature. The BM3 microsatellite (Gaitán-Solís

et al., 2002) was blasted against the Phytozome *Phaseolus vulgaris* v.2.1 genome (<https://phytozome.jgi.doe.gov/pz/portal.html>) and showed homology to a transcription factor B3/auxin response factor of Arabidopsis (ARF3 gene, e-Value 9.2e-3). It was positioned on chromosome Pv6. The ARF3 gene plays an important role in floral meristem maintenance and it is important for gene expression in response to drought stress during early flower development (Zheng et al., 2018).

The BM189 microsatellite was also blasted against the *Phaseolus vulgaris* v.2.1 genome and showed homology to Dof domain (zinc finger, e-Value 9.2e-3) and was aligned on chromosome Pv3 at Phytozome. The DNA-binding one zinc finger (Dof) family transcription factors (TF) are involved in seed development, regulation of metabolism and stress response (Noguero et al., 2013).

Table 2. QTLs identified from the water stress treatment after CIM analyses using the AND-277×SEA5 population map (*Phaseolus vulgaris* L.) and SSR-SNP markers.

Traits	QTL	Chromosome	Interval (cM)	Marker	LOD	Threshold	Additive Effect	R (%)
Leaf area (cm ² plant ⁻¹)	LA1.1 ^{AS}	Pv01	111.7-140.7	PVBR3	6.52	3.15	-0.70	20.30
Root length (cm plant ⁻¹)	RL1.1 ^{AS}	Pv01	13.7-156.1	ATA3	3.19	3.09	-3.12	9.75
Root length (cm plant ⁻¹)	RL6.1 ^{AS}	Pv06	0-34	BM3	3.28	3.09	4.37	19.20
Root superficial area (cm ² plant ⁻¹)	RSA6.1 ^{AS}	Pv06	0-57	BM3	5.18	3.00	0.33	32.60

Table 3. QTLs identified from the well-watered treatment after CIM analyses using the AND-277×SEA5 population map (*Phaseolus vulgaris* L.) and SSR-SNP markers

Trait	QTL	Chromosome	Interval	Marker	LOD*	Threshold	Additive Effect	R(%)
Stem biomass (fresh)	SBF1.1 ^{AS}	Pv01	111.7-140.7	PvBR3	7.67	3.20	-0.12	21.73
Stem biomass (fresh)	SBF3.1 ^{AS}	Pv03	6.3-33	BM189	3.7	3.20	0.08	9.28
Stem biomass (fresh)	SBF3.2 ^{AS}	Pv03	50.9-70.9	BAR5192	3.5	3.20	0.12	15.88
Stem biomass (fresh)	SBF3.3 ^{AS}	Pv03	82.6-108.2	BAR3353	5.12	3.20	-0.12	15.77
Stem biomass (fresh)	SBF6.1 ^{AS}	Pv06	38.1-57	BAR4089	4.14	3.20	0.09	10.99
Leaf biomass (fresh)	LBF1.1 ^{AS}	Pv01	107-140.7	PvBR3	8.92	3.15	-0.26	26.67
Leaf biomass (fresh)	LBD1.1 ^{AS}	Pv01	111.7-140.7	BAR4423	5.18	3.12	-0.13	16.80
Root Length	RL2.1 ^{AS}	Pv02	50.6-72.7	PvBR25	5.58	3.21	-6.33	19.20
Root Volume	RV6.1 ^{AS}	Pv06	0-49	FJ20	3.31	2.99	0.40	16.60
Root superficial area	RSA1.1 ^{AS}	Pv01	111-140	PvBR3	5.02	3.14	-1.96	18.60

The bredline SEA 5 had significantly better values for both shoot and root traits than the other genotypes, except for stem biomass (dry), under WS treatment. It behaved similarly when exposed to WW conditions, except for root length and root superficial area, because root growth was higher with WS treatment. AND 277 had a significantly reduced leaf area (LA), leaf biomass (fresh, LBF), root length (RL), root superficial area (RSA), and root volume (RV) after the WS treatment, whereas SEA 5, under WS treatment, had similar values for these traits for both WW and WS treatments.

Broad sense heritability values varied among the traits and treatments, but they were relatively moderate overall (Table 2). Chlorophyll levels in the WW treatment and root surface area in the WS treatment had the highest h^2 values at 0.85 and 0.82, respectively, while leaf biomass (fresh, LBF), stem biomass (fresh, SBF), and root diameter (RD) had the lowest h^2 values (0.05, 0.01, and 0.07) after the WS treatment. The latter are more likely to be affected by drought stress than chlorophyll and root superficial area. Moreover, as chlorophyll and stem biomass (dry) exhibited no contrasting difference

between AND277 and SEA5 (parents) in both (WS and WW) treatments they were not mapped; however, RILs for both traits portrayed transgressive segregation pattern.

The WS treatment results showed that the SEA 5 genotype outperformed the other genotypes. For example, the AND 277 leaf area decreased by 70% under WS treatment, whereas the SEA 5 leaf area decreased only by 15%, which showed that it was more tolerant to soil drying. The AND 277 leaf biomass (fresh) decreased by 75% compared with 36% for SEA 5. Total root length for SEA 5 was almost twice as long under WS treatment than it was under the WW conditions, whereas the AND 277 root length values decreased by 50%. Previous studies showed that SEA 5 had a profound primary root that grows vertically downward and gives off small lateral roots (CIAT, 2004). In under drought conditions, it has rapid root growth and high grain yield due to vigorous root system and superior ability to mobilize photosynthates (Polania et al., 2017a) which may have played a key role in its superior yield performance (White and Castillo, 1992).

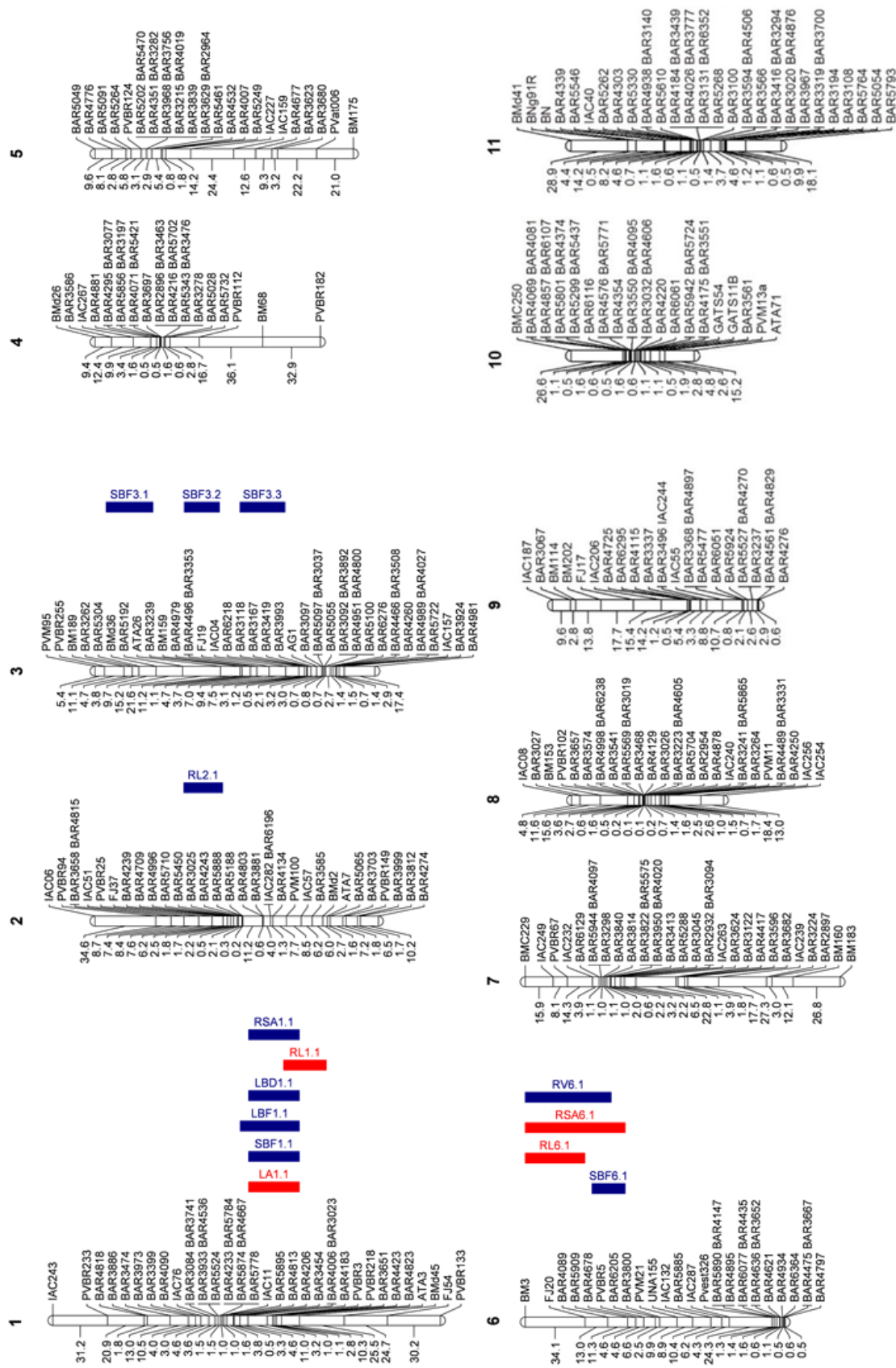


Figure 2. QTL positions in the well-watered (blue) and water-stress treatment plants (red). Chromosomes were assigned using the *P. vulgaris* L. genome (<http://www.phytozome.net/>). The full common bean linkage map (AND-277xSEA5) was published in Brítez et al. (2017). Abbreviations of the OTL are as referred in Tables 2 and 3.

These results confirm the results reported by Asfaw and Blair (2012), Briñez et al. (2017) and Polania et al. (2017a), who also suggested that SEA 5 had superior adaptation to drought stress.

Deep rooting ability has been shown to be positively associated with improved adaptation to drought (Polania et al., 2016; Lynch, 2018). Studies on root traits of chickpea, common bean, soybean, and cowpea indicated that root length, density, depth, and a greater size of root system, could improve drought resistance (Farooq et al., 2017).

To date, there are no models available that can evaluate specific phenes and their states at the required level of detail, or that can faithfully model properties that have emerged due to soil-root-shoot connections (Tardieu et al., 2017). However, the rhizotron approach is a non-destructive method that enables to monitor root development at different soil depths. It may be used to follow root growth dynamics and to quantify differences in root traits. These underground traits are difficult to evaluate in the field, especially when there are large numbers of genotypes and/or treatments. The method also allows yield and other traits related to crop performance to be correlated under water deprivation.

Although the types of adaptations to the water deficit are not always specific and many plants have one or more adaptations that increase tolerance to water deficit, in this study QTLs of greater effect were identified under WS conditions. Drought is becoming a serious problem because of climate change. Drought involves many genes, which, individually, produce limited effects (Briñez et al., 2017).

The fact that there were QTLs which were in the same marker interval suggests that there may be QTL clusters with pleiotropic effects. Microsatellites may be important tools to select bean cultivars with desired root system (length and area in different soil layers) under water deficit regimes.

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References

- ASFAW, A.; BLAIR, M.W. 2012. Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. **Molecular Breeding**, 30 (2):681-695. <https://doi.org/10.1007/s11032-011-9654-y>
- BEEBE, S.E.; RAO, I.M.; BLAIR, M.W.; ACOSTA-GALLEGO, J.A. 2013. Phenotyping of common beans for adaptation to drought. **Frontiers in Physiology**, 4:1-19. <https://doi.org/10.3389/fphys.2013.00035>
- BLAIR, M.W.; CHAVARRO, C.M.; MUÑOZ-TORRES, M.C.; GIRALDO, M.C.; PEDRAZA, F. 2009. Development and diversity of Andean-derived, gene-based microsatellites for common bean (*Phaseolus vulgaris* L.). **BMC Plant Biology**, 9(1):100. <https://doi.org/10.1186/1471-2229-9-100>
- BRIÑEZ, B; PERSEGUINI, J.M.K.C.; ROSA, J.S.; BASSI, D.; GONÇALVES, J.G.R.; ALMEIDA, C; PAULINO, J.F.C.; BLAIR, M.W.; CHIORATTO, A.F.; CARBONELL, S.A.M.; VALDISSER, P.A.M.R.; VIANELLO, R.P.; BENCHIMOL-REIS, L.L. 2017. Mapping QTLs for drought tolerance in a SEA 5 x AND 277 common bean cross with SSRs and SNP markers. **Genetics and Molecular Biology**, 40(4):813-823. <https://doi.org/10.1590/1678-4685-gmb-2016-0222>
- CAMPOS-VEJA, R.; LOARCA-PINA, G.; OOMAH, B.D. 2010. Minor components of pulses and their potential impact on human health. **Food Research International**, 43(2):461-482. <https://doi.org/10.1016/j.foodres.2009.09.004>

- CIAT. 2004. Annual Report. Available at: <http://www.ciat.cgiar.org/beans/pdfs/output_1_04.pdf>
- CIMMYT. 2005. Laboratory protocols. **CIMMYT Applied Molecular Genetics Laboratory**, 3rd ed, Mexico, 81 p.
- FAROOQ, M.; GOGOI, N.; BARTHAKUR, S.; BAROOWA, B.; BHARADWAJ, N.; ALGHAMDI, S.S.; SIDDIQUE, K.H.M. 2017. Drought stress in grain legumes during reproduction and grain filling. **Journal of Agronomy and Crop Science**, 203(2):81-102. <https://doi.org/10.1111/jac.12169>
- GAITÁN-SOLÍS, E.; DUQUE, M.C.; EDWARDS, K.J.; TOHME, J. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris*): Isolation, characterization and cross-species amplification in *Phaseolus* ssp. **Crop Science**, 42: 2128-2136. <https://doi.org/10.2135/cropsci2002.2128>
- GONÇALVES-VIDIGAL, M.C.; CRUZ, A.S.; GARCIA, A.; KAMI, J.; VIDIGAL-FILHO, O.S.; SOUSA, L.L.; MCCLEAN, P.; GEPTS, P.; PASTOR-CORRALES, M.A. 2011. Linkage mapping of the Phg-1 and Co-1⁴ genes for resistance to angular leaf spot and anthracnose in the common bean cultivar AND 277. **Theoretical and Applied Genetics**, 122(5):893-903. <https://doi.org/10.1007/s00122-010-1496-1>
- HAMMER, Ø.; HARPER, D.A.T.; RYAN, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. **Paleontologia Electronica**, 4(1):9. Available at: <https://palaeo-electronica.org/2001_1/past/past.pdf>
- LANNA, A.C.; SILVA, R.A.; FERRARESI, T.M.; MENDONÇA, J.A.; COSTA-COELHO, G.R.; MOREIRA, A.S.; RIBEIRO-VALDISSER, P.A.M.; BRONDANI, C.; VIANELLO, R.P. 2018. Physiological characterization of common bean (*Phaseolus vulgaris* L.) under abiotic stresses for breeding purposes. **Environmental Science and Pollution Research**, 25(31):31149-31164. <https://doi.org/10.1007/s11356-018-3012-0>
- LYNCH, J.P. 1995. Root architecture and plant productivity. **Plant Physiology**, 109(1):7-13. <https://doi.org/10.1104/pp.109.1.7>
- LYNCH, J.P. 2018. Rightsizing root phenotypes for drought resistance. **Journal of Experimental Botany**, 69(13):3279–3292. <https://doi.org/10.1093/jxb/ery048>
- MARGARIDO, G.R.A.; SOUZA, A.P.; GARCIA, A.A.F. 2007. OneMap: software for genetic mapping in outcrossing species. **Hereditas**, 144(3): 78-79. <https://doi.org/10.1111/j.2007.0018-0661.02000.x>
- MUKESHIMANA, G.; BUTARE, L.; CREGAN, P.; BLAIR, M.W.; KELLY, J.D. 2014. Quantitative trait loci associated with drought tolerance in common bean. **Crop Science**, 54(3): 923-938. <https://doi.org/10.2135/cropsci2013.06.0427>
- MÜLLER, B.S.F.; PAPPAS, G.J.; VALDISSER, P.A.M.R.; COELHO, G.R.C.; DE MENEZES, I.P.P.; ABREU, A.G.; BORBA, T.C.O.; SAKAMOTO, T.; BRONDANI, C.; BARROS, E.G.; VIANELLO, R.P. 2015. An operational SNP panel integrated to SSR markers for the assessment of genetic diversity and population structure of the common bean. **Plant Molecular Biology Reporter**, 33(3): 1697-1711. <https://doi.org/10.1007/s11105-015-0866-x>
- NOGUERO, M.; ATIF, R.M.; OCHATT, S.; THOMPSON, R.D. 2013. The role of the DNA-binding One Zinc Finger (DOF) transcription factor family in plants. **Plant Science**, 209: 32–45. <https://doi.org/10.1016/j.plantsci.2013.03.016>

- PAEZ-GARCIA, A.; MOTES, C.M.; SCHEIBLE, W.-R.; CHEN, R.; BLANCAFLOR, E.B.; MONTEROS, M.J. 2015. Root Traits and Phenotyping Strategies for Plant Improvement. **Plants**, 4(2):334-355. <https://doi.org/10.3390/plants4020334>
- PÉREZ VEGA, J.C.; BLAIR, M.W.; MONSERRATE, F.; LIGARRETO, G. 2011. Evaluation of an Andean common bean reference collection under drought stress. **Agronomía Colombiana**, 29(1):17-26. http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-99652011000100003&lng=en&tlng=en.
- POLANIA, J.; RAO, I.M.; CAJIAO, C.; RIVERA, M.; RAATZ, B.; BEEBE, S. 2016. Physiological traits associated with drought resistance in Andean and Mesoamerican genotypes of common bean (*Phaseolus vulgaris* L.). **Euphytica**, 210(1):17-29. <https://doi.org/10.1007/s10681-016-1691-5>
- POLANIA, J.; RAO, I.M.; CAJIAO, C.; GRAJALES, M.; RIVERA, M.; VELASQUEZ, F.; RAATZ, B.; BEEBE, S.E. 2017a. Shoot and root traits contribute to drought resistance in recombinant inbred lines of MD 23-24 x SEA 5 of common bean. **Frontiers in Plant Science**, 8:296. <https://doi.org/10.3389/fpls.2017.00296>
- POLANIA, J.; POSCHENRIEDER, C.; RAO, I.M.; BEEBE, S. 2017b. Root traits and their potential links to plant ideotypes to improve drought resistance in common bean. **Theoretical and Experimental Plant Physiology**, 29(2):143-154. <https://doi.org/10.1007/s40626-017-0090-1>
- RAO, I.M. 2014. **Advances in improving adaptation of common bean and Brachiaria forage grasses to abiotic stresses in the tropics**. In: Handbook of Plant and Crop Physiology. (Ed. M Pessaraki) pp. 847-889. CRC Press, 3rd ed, Boca Raton, Florida. <https://hdl.handle.net/10568/35000>
- RAO, I.; BEEBE, S.; POLANIA, J.; RICAURTE, J.; CAJIAO, C.; GARCIA, R.; RIVERA, M. 2013. Can tepary bean be a model for improvement of drought resistance in common bean? **African Crop Science Journal**, 21(4):265-281.
- RAO, I.M.; BEEBE, S.E.; POLANIA, J.; GRAJALES, M.; CAJIAO, C.; RICAURTE, J.; GARCIA, R.; RIVERA, M. 2017. Evidence for genotypic differences among elite lines of common bean in the ability to remobilize photosynthate to increase yield under drought. **Journal of Agricultural Science**, 155(6):857-875. <https://doi.org/10.1017/S0021859616000915>
- SINGH, S.P.; GEPTS, P.; DEBOUCK, D.G. 1991. Races of common bean (*Phaseolus vulgaris*, Fabaceae). **Economic Botany**, 45(3):379-396. <https://doi.org/10.1007/BF02887079>
- SINGH, S.P. 1995. Selection for water stress tolerance in interracial populations of common bean. **Crop Science**, 35(1):118-124. <https://doi.org/10.2135/cropsci1995.0011183X003500010022x>
- SINGH, S.P.; TERÁN, H.; GUTIERREZ, J.A. 2001. Registration of SEA 5 and SEA 13 drought tolerant dry bean germplasm. **Crop Science**, 41(1):276-7. <https://doi.org/10.2135/cropsci2001.411276x>
- SINGH, S.P. 2007. Drought resistance in the race Durango dry bean landraces and cultivars. **Agronomy Journal**, 99(5):1219-1225. <https://dx.doi.org/10.2134/agronj2006.0301>
- STROCK, C.F.; BURRIDGE, J.; MASSAS, A.S.F.; BEAVER, J.; BEEBE, S.; CAMILO, S.A.; FOURIE, D.; JOCHUA, C.; MIGUEL, M.; MIKLAS, P.N.; MNDOLWA, E.; NCHIMBI-MSOLLA, S.; POLANIA, J.; PORCH, T.G.; ROSAS, J.C.; TRAPP, J.J.; LYNCH, J.P. 2019. Seedling root architecture and its relationship with seed yield across diverse environments in *Phaseolus vulgaris*. **Field Crops Research**, 237:53-64. <https://doi.org/10.1016/j.fcr.2019.04.012>

- SUBBARAO, G.V.; JOHANSEN, A.C.; SLINKARD, R.C.; RAO, N.; SAXENA, N.P.; CHAUHAN, Y.S. 1995. Strategies for improving drought resistance in grain legumes. **Critical Review in Plant Science**, 14(6):469-529. <https://doi.org/10.1080/07352689509701933>
- TARDIEU, F.; VARSHNEY, R.K.; TUBEROSA, R. 2017. Improving crop performance under drought-cross-fertilization of disciplines. **Journal of Experimental Botany**, 68(7):1393-1398. <https://doi.org/10.1093/jxb/erx042>
- WHITE, J.W.; CASTILLO, J.A. 1992. Evaluation of diverse shoot genotypes on selected root genotypes of common bean under soil water deficits. **Crop Science**, 32(3):762-765. <https://doi.org/10.2135/cropsci1992.0011183X003200030037x>
- ZHENG, Y.; ZHANG, K.; GUO, L.; LIU, X.; ZHANG, Z. 2018. Auxin response factor 3 plays distinct role during early flower development. **Plant Signaling & Behavior**, 13(5):1-4. <https://doi.org/10.1080/15592324.2018.1467690>