Soil Compaction Induced Changes in Morpho-physiological Characteristics of Common Bean

Mariela Rivera¹ · José Polanía¹ · Jaumer Ricaurte¹ · Gonzalo Borrero¹ · Stephen Beebe¹ · Idupulapati Rao^{1,2}

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Abstract

The progress in identification of genotypes with roots showing greater ability to penetrate compacted soils under moisture stress and understanding the physiological basis and morphological expression of the characteristics associated is fundamental to improving crop's tolerance to drought and to increase productivity under individual and combined stress conditions. Our objectives were to: (i) determine genotypic differences in root penetration ability of common bean (*Phaseolus vulgaris* L.) using two methods (compacted soil layers and wax-petrolatum layers) to simulate soil compaction; and (ii) identify the morpho-physiological characteristics associated with the ability to penetrate compacted layers under well-watered and intermittent drought conditions in a greenhouse environment. Six common bean genotypes of different origin (Andean, Mesoamerican and interspecific) were tested in two trials. In the first trial, we evaluated genotypic differences in morpho-physiological characteristics that were associated with the root's ability to penetrate compacted soil layers of 1.2, 1.4, and 1.6 g cm⁻³ bulk densities. In the second trial, we used another method to determine genotypic differences in root's ability to penetrate wax-petrolatum layers of low, medium, and high mechanical impedance. The conditions for root growth were severely restricted by compaction and aggravated by drought stress. The medium level of mechanical impedance of wax petrolatum layer method showed the best results to evaluate bean genotypes for soil compaction tolerance. Among the six genotypes tested, ALB 91 (an interspecific line) was identified as the one with the greatest root penetration ability compared with A774 which showed the lowest root penetration ability under both well-watered and drought conditions.

Keywords Abiotic stress · Drought · Root hair length · Root penetration · Soil bulk density · Wax-petrolatum layers

1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for human consumption and it is grown by small farmers in marginal areas and with minimal use of inputs (Thung and Rao 1999; Beebe et al. 2013). Many of the soils used for production of common bean, besides being prone to drought, suffer from some degree of soil compaction (Buttery

et al. 1998; Thung and Rao 1999). The development of common bean cultivars adapted to drought conditions in compacted soils is an important strategy that contributes to food security in the face of climate change. Identification of key plant traits and understanding physiological mechanisms that contribute to improved drought adaptation in common bean can increase the efficiency of breeding programs through selection of superior genotypes for these soil conditions (Beebe et al. 2013).

Soil compaction is a major constraint to agricultural production and is primarily caused by wheel traffic from heavy equipment (often when operations are conducted on wet soils) due to a reduction in soil mechanical strength (White and Kirkegaard 2010; Chimungu et al. 2015). Soil compaction affects porosity, pore connectivity, infiltration, air permeability, temperature, rooting space, nutrient flow, and soil biological activity (Kozłowski 1999; Khan et al. 2017). It also limits plant growth and development

✉ Idupulapati Rao
i.rao@cgiar.org

¹ International Center for Tropical Agriculture (CIAT), A. A. 6713, Recta Cali-Palmira Km 17-CIAT, Cali, Colombia

² Present address: Plant Polymer Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 North University Street, Peoria, IL 61604, USA

as it reduces the extraction of water and nutrients (Grzesiak et al. 2017; Descalzi et al. 2018).

Increased soil strength and limited aeration are likely to be the two main physical factors that affect plant growth in compacted soils (Kozłowski 1999; Burr-Hersey et al. 2017). Mechanical impedance decreases the rate of plant cell division and reduces the cell length in the root meristem (Bengough et al. 2011) reducing rooting density and depth.

The increased ability of the roots to penetrate compacted soils can be beneficial to avoid drought stress through deep rooting (Yu et al. 1995). Some morpho-physiological root traits that help plants to deal with soil compaction by improving penetration is to enhance root diameter, increase border cells' release, secretion of mucilage at the root tip, and stiffening of cell walls (Bengough et al. 2011; McKenzie et al. 2013). The formation of root cortical aerenchyma, reduction of cortical cell file number, reduction of cortical cell size, and proliferation of longer root hairs closer to root tips for anchorage (Lynch and Wojciechowski 2015) facilitate growth by reducing metabolic burden and oxygen requirement. This allows deeper soil penetration and potentially greater hypoxia tolerance. Several researchers have reported genotypic variation in the ability of cereal roots to penetrate wax barriers in the laboratory, which is often correlated with rooting depth and crop performance under field conditions (Botwright Acuña et al. 2007). In the field, significant genotype by environment interaction ($G \times E$) occurs for these traits with major implications when designing better breeding strategies (Botwright Acuña et al. 2007; Botwright Acuña and Wade 2012).

We tested the hypothesis that common bean genotypes differ in their root penetration ability through compacted soil layers under adequate soil moisture (well-watered) and also water stress (drought) conditions. We simulated soil compaction using two methods (compacted soil layers and wax-petrolatum layers) to identify a suitable evaluation method that can be used in a common bean genetic improvement program to select superior genotypes.

The main objectives of this study were to: (i) determine genotypic differences in root penetration ability of six common bean genotypes using two methods to simulate soil compaction; and (ii) identify the morpho-physiological characteristics associated with the ability to penetrate compacted layers under well-watered (80% of field capacity) and intermittent drought conditions (50% of field capacity) in a greenhouse environment.

2 Materials and Methods

This study comprised two experiments conducted using the greenhouse facilities of the International Center for Tropical

Agriculture CIAT located in Palmira (3° 31' N and 76° 19' W, at an altitude of 965 m.a.s.l.), Valle del Cauca, Colombia.

2.1 Experiment 1: Use of Dense Soil Layers to Identify Characteristics Associated with Root Penetration in Compacted Soils

2.1.1 Experimental Design and Treatments

This trial was conducted with treatments distributed as a split-split plot design with four replications in August 2013 and included the following treatments: the main plots were two levels of water supply (well-watered and intermittent drought), the sub-plots were six genotypes of common bean, and the sub-sub plots were three levels of compaction. The six genotypes used from CIAT's breeding program were: A774, ALB 91, BAT 477, CALIMA, DAB 295, and SMC 140. ALB 91 is an interspecific line developed with introgression of *Phaseolus coccineus* genes into *Phaseolus vulgaris* (Butare et al. 2012). Three soil compaction treatments were imposed for each genotype under each level of water supply. For each level of water supply and for each genotype, soil cylinders (0.80 m length, 0.10 m diameter, and 6.3 L of capacity per cylinder) with 10 cm of soil layer just 5 cm below the soil surface was uniformly compacted at the following three soil bulk densities (BD): 1.2, 1.4, and 1.6 g cm⁻³. These three BD values to simulate different levels of soil compaction were selected based on the published reports (Buttery et al. 1998; Horn and Fleige 2009; Reynolds et al. 2009) and also from preliminary experiments conducted by the authors where the value of 1.2 did not show any restriction of root growth while the value of 1.4 showed moderate effect of compaction, and 1.6 showed larger effect of compaction. Water loss through soil surface from each soil cylinder was monitored by maintaining soil cylinders with no plants under each level of water supply. All plants were inoculated with *Rhizobium* strain CIAT 899 to stimulate symbiotic nitrogen fixation.

2.1.2 Soil Column Preparation and Establishment of Plants

The soil used in the columns was taken from a Mollisol Ap horizon (Aquic Hapludoll) with no major fertility problems (pH 7.7) and it was obtained from CIAT, Palmira. The soil was sieved and mixed with washed river sand in 2:1 w/w proportions. Plants were cultivated in cylindrical polyethylene soil tubes (0.80 m long, 0.10 m internal diameter, and 6.3 L capacity) that were inserted into thin walled PVC tubes (Butare et al. 2012) to assess the development and distribution of roots in soil cylinders. We used a hydraulic press to establish different pressures with predetermined soil quantities to a fixed volume to create three different soil bulk densities. The pressures were uniformly applied according to each BD value. Soil compaction was simulated by using previously

compacted soil layers and not the complete amount of soil in the column.

For treatments with 1.2 g cm^{-3} bulk density, polyethylene cylinders were filled with soil and sand mixture at 77 cm of height. Whereas for the treatments of 1.4 and 1.6 g cm^{-3} BD, previously compacted layers were placed into the cylinders that contained the initial mixture of uncompacted soil at 80% field capacity (soil column of 0.62 m depth), and then brought to 0.77 m by the addition of compacted soil layer (10 cm) and uncompacted soil (5 cm). Intermittent drought stress was induced at 12 days after planting and the application of water in the intermittent drought treatments was restarted 12 days after stress induction and maintained at 50% field capacity (FC) throughout the experiment. Soil moisture was maintained at 80% (well-watered treatment) and 50% field capacity (intermittent drought stress treatment) by weighing each cylinder every 2 days and applying water to the soil at the top of the cylinder. Field capacity was determined based on volumetric water content derived from a water retention curve (Richards 1965). The value was verified also by determining the gravimetric soil water retention of five soil tubes after fully drained condition. The plants were harvested at 45 days after planting corresponding to the R8 growth stage of mid-pod filling.

2.1.3 Plant Attributes Measured

The following plant attributes were measured to identify the morpho-physiological characteristics that are associated with tolerance to soil compaction and its interaction with drought stress based on their degree of phenotypic association. Leaf chlorophyll content (non-destructive) was determined with a hand-held SPAD meter (SPAD-502 Minolta Camera Co., Ltd., Japan) on young and fully expanded leaves and the results are reported as SPAD units. Stomatal conductance was determined using a porometer (Decagon Devices, Inc. www.decagon.com) and was measured between 10:00 and 11:30 in the morning and the results are reported as $\text{mmol m}^{-2} \text{ s}^{-1}$. Photosynthetic efficiency (photosystem II in light adapted leaves) was measured using a FP100 Fluorpen on a fully expanded young leaf and was expressed in real or effective quantum yield (F_v/F_m'). Concentrations of non-structural carbohydrates (Kang and Bringk 1995) and phosphorus (P) in shoot and root biomass were determined using a spectrophotometer. Leaf area was measured with a leaf area meter (LI-3000 Model from LI-COR). Total biomass was measured at harvest by separating different plant components and drying them at 60°C for 48 h to determine dry weight. Plant roots collected from each soil cylinder were washed manually and scanned (Epson Expression 10000XL), and total root length (m plant^{-1}), mean root diameter (mm), and root volume (g cm^{-3}) were determined using a WinRHIZO image analysis system (Butare et al. 2012). The length and density of root

hairs were measured using a scanner. A group of ten seeds per genotype was pregerminated in a sandwich system (foam, germination paper, seeds, germination paper, foam), the system was partially submerged in a container with a sheet of water of 4 cm for 5 days. Three seedlings were selected, in each of them, two sections were demarcated with respect to the base for both the main root and the basal root of the second whorl. The first section was between 0.0 and 0.5 cm and the second section was between 2.0 and 2.5 cm. In each demarcated section, six sites were scanned to obtain images of root hairs and then analyzed using the software WinRhizo 2007d, to determine the length of root hairs.

2.1.4 Data Analysis

Analysis of variance of the data was performed using a PROC ANOVA procedure of the SAS system (SAS Institute Inc., Cary, NC, USA) and correlations were calculated with the PROC CORR procedure (Pearson) of the SAS System (SAS Institute, Inc. 2012). Data shown are mean values for each environment (well-watered or drought), bulk density (BD) treatment, and genotype (G). The least significant difference (LSD) was calculated to compare the mean values. Values marked with *, **, and *** are statistically significant at probability levels of 5%, 1%, and 0.1%, respectively. The significance of the interaction effects of $E \times BD \times G$ was also tested using the same software.

2.2 Experiment 2: Use of Wax-Petrolatum Layers to Screen Common Bean Genotypes for Differences in Root Penetration Ability

2.2.1 Experimental Design and Treatments

This experiment was established under similar environmental conditions, soil type, moisture management, and genotypes used in the first experiment. This experiment was also carried out as a split-split-plot design with three replications in the month of October 2013. The main plots were two levels of water supply (well-watered and drought induced by progressive soil drying), the sub-plots were six genotypes of common bean (*Phaseolus vulgaris* L.), and the sub-sub plots were three levels of mechanical impedance created by wax-petrolatum layers. The experimental units consisted of acetate cylinders of 10 cm diameter, 20 cm high, and 2.2 L capacity. The cylinders were filled to a height of 15 cm with the soil and sand mixture, then the paraffin disk was introduced and completed with more soil and sand mixture 2 cm from the surface, and each cylinder was covered with cardboard. At 20 days after planting, the number of roots that penetrated the lower surface of the each wax-petrolatum layer was counted.

Then the soil was rinsed of the root mass and the total number of roots was counted.

2.2.2 Wax Petrolatum Layers

The wax-petrolatum layers consisted of 3.5-mm thick disks made of wax and white petrolatum mixtures in three proportions: (a) 20% and 80%, (b) 60% and 40%, and (c) 80% and 20%, equivalent to a low, media, and high mechanical impedance with a penetration resistance strength of 0.025, 1.2, and 1.8 MPa, respectively at 24 °C and with similar moisture content. The mechanical resistance of each wax and white petrolatum mixture was determined using a soil penetrometer (DIK-5561, Daikirikakogyo Co., Ltd. Tokyo, Japan) at room temperature. The wax and white petrolatum mixture was heated to 76 °C and the molten mixture was kept on a magnetic stirrer for 15 min until the wax was completely melted. Immediately after, the mixture was poured in 30 ml proportions into acrylic cylinder molds (3.5-mm height and 10-cm internal diameter). The mixture was left to solidify at room temperature for 5 min and was then removed from the mold and stored at 4 °C until it was used for the experiment.

2.2.3 Plant Attributes Measured

Penetration ability was evaluated as root penetration ratio, i.e., the number of roots that penetrated the wax-petrolatum layer and the total number of roots per plant (Materechera et al., 1992). The roots were scanned and then analyzed with the WINRhizo software.

2.2.4 Data Analysis

Data collected were analyzed with the SAS System (SAS Institute, Inc., 2012) as described for Experiment 1.

3 Results

3.1 Use of Dense Soil Layers to Identify Characteristics Associated with Root Penetration in Compacted Soils

3.1.1 Response of Morpho-Physiological Traits to Soil Compaction and Drought Stress

Intermittent drought as an environment (E) and increased soil bulk density (BD) affected significantly several morpho-physiological traits of six common bean genotypes (G) evaluated. It was observed that $G \times BD$ interaction accounted for 68% of the variation in pod biomass which was more than three times higher than that attributed to G alone under drought conditions (Table 1). However, pod weight under well-watered conditions was more influenced by G than by

$G \times BD$ (Table 1). For shoot biomass, the $G \times BD$ interaction accounted for 44% and 64% of the variation under well-watered and drought conditions, respectively, indicating a greater interaction effect than with G alone effect (Table 1), evidencing that the compaction aggravates the effects of drought stress (Table 1).

3.1.2 Effects on Shoot and Root Biomass

Soil compaction and drought stress affected drastically the dry matter partitioning between shoot and roots among the genotypes evaluated. Soil compaction and drought stress together reduced the dry matter partitioned to the roots relative to the shoot (Fig. 1). On average, drought stress reduced more drastically the shoot biomass (67.5%) than the root biomass (36.5%) ($P < 0.01$). This differential reduction in shoot biomass resulted in an increase of 52.9% in root:shoot ratio under drought than in well-watered conditions.

Root:shoot ratio was greater in drought and it increased under higher soil BD treatment. Lower shoot biomass reduction due to drought and compaction interaction was observed with three genotypes: Calima, DAB 295, and SCM 140; however, the SMC 140 genotype had the lowest reduction in pod biomass with greater reduction in root biomass under drought conditions, possibly due to greater mobilization of photosynthates to pod formation instead of root biomass accumulation. This genotype (SMC 140) showed tolerance to both drought and soil compaction.

3.1.3 Changes in Non-structural Carbohydrate Levels

The combined analysis showed highly significant differences in concentration of total non-structural carbohydrates (TNC) in shoot biomass between environment, genotypes and soil BD. As expected, reduced shoot and root growth due to compaction and drought interaction also affected the concentration of TNC. The interaction between drought and soil compaction increased the concentration of TNC in shoot biomass most likely due to a cumulative effect in less tissue formation (Fig. 2). Drought stress reduced the concentration of TNC by 42.8%. Genotypes that reduced shoot biomass with higher TNC levels were ALB 91 and BAT 477, whereas A 774 and SMC 140 decreased it to a lesser extent. Under drought conditions TNC concentrations in shoot biomass were negatively correlated with root biomass (-0.39 , $P = 0.03$) indicating that root biomass increased due to drought stress. Drought-resistant genotypes seem to increase mobilization of carbon. An important effect of soil compaction on shoot TNC concentration was observed and this effect could explain 65% of variance under well-watered and drought conditions (Table 1).

Table 1 Variance component proportions by soil BD, G and BD $\times$ G interaction for different morpho-physiological traits under well-watered and drought conditions (data are presented as the percentage of total ratio of the sum of squares)

Sources	PB	SLW	NODB	RB	PSH	TNCH	SHB	MRD	PR	TNCR
Well-watered										
Bulk density (BD)	10**	14	51***	27*	27*	68***	32*	33**	5	24
Genotype (G)	65***	71***	16*	17	50*	14	24	17	47*	54*
G $\times$ BD	25**	15	33*	56*	23	18	44*	50*	48*	22
Drought										
Bulk density (BD)	11	11	52*	18	50***	67***	19	78***	12	14
Genotype (G)	21	74***	11	24	26*	14	17	10	24	15
G $\times$ BD	68*	15	37	58*	24	18*	64*	12	64*	56*

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

PB pod biomass, SLW specific leaf weight, NODB nodule biomass, RB root biomass, PSH phosphorus concentration in shoot, TNCSH total non-structural carbohydrates in shoot, SHB shoot biomass, MRD mean root diameter, PR phosphorus concentration in root, TNCR total non-structural carbohydrates in root

Increases in soil BD increased the shoot TNC concentration with increases of 43% and 31% under well-watered and drought conditions, respectively (Fig. 2). If TNC increases in shoot biomass and if it is an indicator of stress tolerance, then the two genotypes ALB 91 and A774 have better performance in compacted soils under well-watered and drought conditions. While the SMC 140 genotype would be considered as

drought-resistant genotype based on the interaction observed between soil compaction and drought (Fig. 2), it also showed a decline in photosynthetic efficiency (9%) that is a feature that contributes to a better tolerance to these two combined abiotic stresses. The Calima genotype was most sensitive to combined stress of soil compaction and drought in contrast with SMC 140.

Fig. 1 Genotypic differences in mean shoot (A) and root (B) biomass production (g plant^{-1}) as influenced by three different values of soil bulk density and two levels of water supply under greenhouse conditions

Fig. 2 Influence of different soil bulk densities and drought stress on mean values of total non-structural carbohydrates (TNC) concentration (mg g^{-1}) in the shoot biomass of six common bean genotypes grown under greenhouse conditions

3.1.4 Changes in Specific Leaf Weight

Specific leaf weight (SLW) varied between 0.014 and 0.023 g cm^{-2} showing the main effect of genotype which explained more than 60% of the variation in both well-watered and drought conditions (Table 1). The genotypic difference was mainly with soil BD of 1.2 g cm^{-3} with higher values of SLW under drought. The ascending order of the genotypes by SLW values was: Calima > DAB 295 > SMC 140 > A774 = ALB91 > BAT 477.

3.1.5 Changes in Root Morphological Responses

Drought stress affected in general all root parameters evaluated, e.g., root length was reduced by 72%. The increase in soil BD from 1.2 to 1.6 g cm^{-3} reduced root length by 49% and 25% under well-watered and drought conditions, respectively. The genotypes with the highest and lowest root length under well-watered conditions were A774 and BAT 477, showing also the lowest and highest mean root diameter, respectively. Genotypes with fine roots have longer roots. The mean root diameter was not significantly different between environments but increased with higher soil BD (Table 1), with less increase under well-watered (21%) than under drought condition (39%). In many drought environments, the top soil dries before the sub-soil, and as drought progresses, roots must explore increasingly deeper soil strata to acquire water.

Interspecific line ALB 91 showed a higher capacity to increase the mean root diameter with increasing soil BD with values of 0.30 and 0.41 mm, respectively, and this was equivalent to a 39% increase. The root hair length showed highly significant differences ($P < 0.0001$) among the six genotypes when evaluated using germination paper under greenhouse conditions. ALB 91 also showed greater average root hair length of 0.46 mm at the base of the tap root. DAB 295 exhibited shorter root hairs with an average value of 0.16 mm (Fig. 3).

3.1.6 Changes in Nodule Number and Nodule Dry Weight

The number of nodules was affected by the environment and soil BD. Nodule dry weight was affected by the environment and soil BD as well as to their interactions (results not shown). Drought restricted almost entirely the production of nodules while under well-watered conditions the number and weight of nodules increased with higher soil BD. The increase was significant compared to the higher soil BD (1.6 g cm^{-3}). Genotypes with the highest number and dry weight of nodules were DAB 295 > CALIMA > ALB 91 and the genotype with less quantity and weight of nodules was BAT 477. The environment and soil BD interaction effect indicate a differential response to nodule dry weight between soil BD and soil moisture conditions (environment), just as between soil BD and genotypes (Fig. 4). Under well-watered conditions, the number of nodules per plant varied between 84 and 223, and dry weight of nodules showed values between 33 and 205 mg per plant as influenced by increase in soil BD values of 1.2 to 1.6 g cm^{-3} . Increase in root diameter increased the surface area per unit root length that is available for nodule formation, resulting in positive correlations between soil BD and nodule dry weight ($r = 0.63$, $P < 0.0001$), and mean root diameter ($r = 0.35$, $P < 0.001$).

4 Root Penetration Differences among Common Bean Genotypes Grown Under Drought Stress

Root penetration is a measure that indicates the relative ability of a plant's root to penetrate a wax layer. A root penetration value of 1 indicates that all roots penetrated the wax layer while a value of 0 means that none of the roots that reached the wax layer were able to penetrate it. The mixtures of wax and white petroleum in 20:80 and

Fig. 3 Images (magnified) of tap roots of six common bean genotypes showing differences in root hair length (mm) at the base of the tap root using germination paper technique under greenhouse conditions. Root image analysis was performed using WinRhizo software

80:20 proportions, respectively, chosen to evaluate extreme mechanical impedance (low and high), presented a negative correlation between penetration ability and mean root diameter. For this reason and based on the wax-layer strength test, the mixture of wax and white petroleum (60% and 40%) with a media impedance of 1.2 MPa to evaluate differences among common bean genotypes in root penetration was chosen to be used. The mechanical impedance media found to be highly suitable to evaluate genotypic differences in tolerance to soil compaction. Drought stress decreased penetration ability but increased mean root diameter (Table 2).

The analysis of variance showed significant differences in penetration ability and mean root diameter regarding genotypes, environment, and their interaction (data not shown). Root penetration ability ranged from 18 to 46% under well-watered conditions and 15 to 41% under

drought conditions. Root thickness increased under drought stress and it ranged from 0.52 to 0.68 mm in well-watered conditions and 0.60 to 0.80 mm in drought conditions and were positively correlated ($r=0.79$, $P=0.0001$ and $r=0.52$, $P=0.02$) with root penetration ability under well-watered and drought conditions, respectively.

The ALB 91 genotype was identified as the one with the greatest root penetration ability with a 2.6 times higher value than A774 which showed the lowest root penetration ability under both well-watered and drought conditions. Genotype SMC 140 was identified as the one with greatest ability to increase root diameter under drought stress with an increase of 26%. However, this genotype showed better adaptation than the other genotypes in terms of its tolerance to combined stress conditions of drought and soil compaction.

Fig. 4 Mean values of nodule number per plant and dry weight (mg) per plant observed with different soil BD (1.2, 1.4, and 1.6 g cm⁻³) treatments and under well-watered conditions in the greenhouse

Table 2 Penetration ratio and root thickness of six common bean genotypes evaluated using a media impedance wax-layer system to simulate compacted soil layers under well-watered and drought conditions

Genotypes	Root penetration ratio ^a		Mean root diameter (mm)	
	Well-watered	Drought	Well-watered	Drought
A774	0.18 (0.02) ^b	0.15 (0.02)	0.52 (0.02)	0.61(0.05)
ALB91	0.46 (0.04)	0.41 (0.03)	0.65 (0.07)	0.72 (0.10)
BAT477	0.23 (0.05)	0.20 (0.04)	0.56 (0.02)	0.71 (0.02)
CALIMA	0.25 (0.05)	0.23 (0.04)	0.52 (0.02)	0.60 (0.02)
DAB295	0.34 (0.04)	0.36 (0.02)	0.68 (0.03)	0.69 (0.05)
SMC140	0.32 (0.03)	0.30 (0.02)	0.59 (0.06)	0.80 (0.05)
Mean	0.30	0.28	0.59	0.69
C.V	15.4	12.8	8.40	9.6
LSD _{0.05}	0.08	0.06	0.09	0.12

^a Mean ratio of the number of roots penetrating the wax layer over the total number of roots

^b Standard error

5 Discussion

5.1 Genotypic Differences in Root Penetration Ability

We found significant differences in penetration ability and mean root diameter regarding genotypes, environment, and their interaction. These results are consistent with those found by Yu et al. (1995) with rice and Chimungu et al. (2015) with maize who used wax-petrolatum layers and found positive and significant correlations between root penetration ability and root diameter. However, Whalley et al. (2013) found no differences in wheat genotypes regarding neither root penetration ability nor root mean diameter when they worked with wax-petrolatum layers.

The ALB 91 genotype was identified as the one with the greatest root penetration ability than A774 which showed the lowest root penetration ability under both well-watered and drought conditions. Root thickness is considered as an important root characteristic in improving drought resistance (Yu et al. 1995), and is also suggested to play a role in penetration ability. Thicker roots are associated with good penetration in strong soils by relieving stress at the growing root tips (Bengough et al. 2011). However, the specific mechanisms by which roots penetrate compacted soil layers are still not well understood.

SMC 140 was outstanding in its ability to increase root diameter under drought stress. Yu et al. (1995) found that root thickening could be an adaptive mechanism to drought stress independent of the penetration ability in compacted layers. However, SMC 140 showed better adaptation than the other genotypes in terms of its tolerance to combined stress

conditions of drought and soil compaction. Results from this study indicate that the wax-petrolatum layers (with a 60:40 wax–white petrolatum proportion) should be an effective mix to be used as a technique to measure differences in root penetration ability among common bean genotypes.

5.2 Identifying Morpho-Physiological Characteristics Associated with Root Penetration in Compacted Soils

Soil compaction and drought stress together reduced the dry matter partitioned to the roots relative to the shoot and this differential reduction in shoot biomass resulted in increase of root:shoot ratio under drought than in well-watered conditions. It is well-known that water stress substantially increases root growth in comparison to shoot growth (Palta and Gregory 1997). A greater root to shoot ratio means that each unit of leaf area has more non-photosynthetic tissue to sustain, reducing the overall plant growth rate. Results on the effect of soil BD values on shoot and root growth responses are in agreement with previous studies on the effects of soil compaction on plant development (Alameda et al. 2012) who reported shoot and root biomass of *Nicotiana tabacum* to increase in soil compaction treatment until a soil BD value of 1.4 g cm⁻³ under well-watered conditions. Moderate soil compaction stress can stimulate plant growth (Alameda and Villar 2009), but these results are contrary to the general concept that soil compaction negatively affects plant performance (Kozłowski 1999). The positive effect is probably due to a better root-soil contact, which improves water and nutrient uptake. Moreover, compaction can increase mass transport flow by increasing hydraulic conductivity (Alameda and Villar 2009). Compaction reduced the proportion of fine roots by increasing the average diameter of the roots (results not shown) probably as an adaptation mechanism to cope with reduced pore space.

We found that root:shoot ratio was greater under drought stress and it increased with higher soil BD treatment. The partitioning of structural material among the various plant organs is determined by their genetic traits, ontogenetic development, and environmental conditions. Genotypic differences were reported in common bean for root biomass, root:shoot ratio, root surface area, root length, and mean root diameter (Butare et al. 2012; Beebe et al. 2013). The success of plants under stress conditions may be determined by their ability to control the use of carbohydrates to generate metabolic energy. Bean lines selected for abiotic stress tolerance also had greater harvest indices and increased seed yield in favorable environments (Beebe et al. 2013).

Although lower shoot biomass reduction due to drought and compaction interaction was observed with three genotypes, SMC 140 genotype showed tolerance to both drought and soil compaction. Nielsen et al. (2001) reported that net assimilation rate is reduced less than leaf area growth

rate under P deficiency so that there is an oversupply of carbohydrates in the shoot and an increase in the translocation of carbon to the root system, thus, promoting root growth. Adapting bean genotypes to soil compaction would be associated with the ability of roots to explore soil volume at a minimum metabolic cost.

Results observed with changes in TNC levels are in agreement with those published by Sala et al. (2010) that showed decrease in the concentration of TNC due to drought. Piper (2011) observed decreasing values of TNC in more drought-susceptible species (*Nothofagus nitida*) while the values were increasing in the more drought-resistant species (*Nothofagus dombeyi*). Under drought conditions, the negative correlation observed between TNC concentrations in shoot biomass with root biomass indicated that root biomass increased due to drought stress. Drought-resistant genotypes seem to increase mobilization of carbon to support root growth. An important effect of soil compaction on shoot TNC concentration was observed and this effect could explain 65% of variance under well-watered and drought conditions.

We found that an increase in soil BD could increase the shoot TNC concentration under both well-watered and drought conditions. Drought-induced accumulation of carbon reserves might be more prone to happen in those genotypes that are better adapted to drought. Thus, the drought resistance of a genotype may be an important factor in determining whether TNC depletion does occur (Piper 2011). If TNC increases in shoot biomass and if it is an indicator of stress tolerance, then the two genotypes ALB 91 and A774 have better performance in compacted soils under well-watered and drought conditions. While the SMC 140 genotype would be considered as drought-resistant genotype based on the interaction observed between soil compaction and drought, it also showed a decline in photosynthetic efficiency (9%) that is a feature that contributes to a better tolerance to these two combined abiotic stresses. Some best drought-adapted plants can down-regulate their carbon demands under drought conditions through physiological responses such as decreased respiration, leaf shedding, and/or limiting growth, as suggested by Piper (2011). We found that Calima genotype was the most sensitive to combined stress of soil compaction and drought in contrast with SMC 140.

Controlled environment experiments with wheat (*Triticum aestivum*) and a field study with sunflower (*Helianthus annuus*) carried out by Wolfe et al. (1995) documented that leaf expansion and growth of young seedlings were significantly reduced in plants grown on compacted soils even when water, oxygen, and nutrients are not limiting factors. Moreover, measurements of net photosynthesis in individual leaves also indicated that growth was not limited by carbohydrate supply. These authors suggested that mechanical impedance of compacted soils may cause a hormonal signal in the roots which could control shoot growth response (Wolfe et al.

1995). Results on changes in SLW were consistent with previous reports by White and Montes (2005) who found that the SLW values varied widely among bean cultivars. Since the SLW of SMC 140 was relatively constant, it is necessary to improve the understanding regarding the interaction of SLW with tolerance to abiotic stress.

Interspecific line ALB 91 showed a higher capacity to increase the mean root diameter with increasing soil BD. ALB 91 also showed greater average root hair length at the base of the tap root. In many drought environments, the top soil dries before the sub-soil, and as drought progresses, roots must explore increasingly deeper soil strata to acquire water. Genotypes capable of supporting greater root biomass would be better conditioned to develop the extensive and deep root system required to use soil water resources fully (Beebe et al. 2013). On the other hand, the sole selection of a genotype for root system size in maize without any regard to costs for building the root system actually decreased drought tolerance by diverting assimilates from grains towards root growth (Bruce et al. 2002).

There is a close relationship between root diameter and the ability of the roots to penetrate compacted horizons. Thick roots relieve the axial stress of the root tips during growth when roots are subjected to stress by mechanical impedance (Bengough et al. 2011). Relieving stress by thick roots depends on the level of soil-root contact which is influenced by the presence of viscous coatings (mucilage) that facilitate slippage (Bengough et al. 2011). Additionally, root diameter and root hair density and length could also play an important role in root penetration through compacted soil horizon.

Haling et al. (2013) tested barley (*Hordeum vulgare*) lines that are with and without root hairs and found that when root hairs were present they were able to penetrate compacted soil. Root hairs improve the anchorage for root tip growth but not necessarily increase the rate of root elongation within the horizon. Another potentially relevant characteristic to root penetration is genotypic variation in relation to the root's ability to reach cracks and channels in the soil (McKenzie et al. 2009).

Drought restricted almost entirely the production of nodules while under well-watered conditions, the number and weight of nodules increased with higher values of soil BD. This observation is consistent with the results reported by Buttery et al. (1994). They found an unexpected increase in the number of nodules per plant in ten common bean genotypes that were evaluated in plots with highly compacted soil (with 1.63 to 1.69 BD values), and they argued that the severe restriction in root growth caused prolonged contact with the inoculum applied to seeds thereby promoting an increase in nodulation. Subsequent trials conducted by them to test this hypothesis failed to reproduce this effect on nodulation (Tu and

Buttery 1988). Although compaction reduced the plant weight increase, it also produced a marked increase in nodule weight as a fraction of the total weight of the plant including an increase in root:shoot ratio of plant biomass. Buttery et al. (1994) suggested that nodulation is probably a function of root growth. Results obtained in this study were similar to those found by Buttery et al. (1998) who reported a decrease in the number of nodules per plant due to the effect of low soil moisture content (11 to 13% for sandy loam soil and 23 to 25% for clay loam soil in terms of available soil moisture (ASM) on volume basis), and an increase in the number of nodules under high soil moisture (20 to 22% for sandy loam soil and 33 to 35% for clay loam soil of ASM on a volumetric basis) by increasing soil BD from 1.2 to 1.6 g cm⁻³. This was also the case in clay loam soil for the common bean genotype A 300 (erect plant type with determinate growth habit, tolerant to root rot, developed at CIAT-Colombia) which was not tested in this study (Buttery et al. 1998). We observed an interaction effect between the environment and soil BD and this indicates a differential response to nodule dry weight between soil BD and soil moisture conditions (environment), just as between soil BD and genotypes.

6 Conclusions

It was possible to evaluate the influence of individual and combined effects of soil compaction and soil moisture regime on root and nodule development and plant growth under greenhouse conditions. The conditions for root growth were severely restricted by soil compaction and aggravated by drought. The difference in response among genotypes under compacted soil conditions and their interaction with drought suggests that it is possible to select and improve genotypes for superior performance in compacted soils in bean growing areas that are prone to drought. Two common bean genotypes (ALB 91, SMC 140) were identified as tolerant to soil compaction and drought stress and their superior performance was associated with greater root penetration ability, thicker root system with longer root hairs. We suggest that wax-petrolatum layers with a mixture of wax and white petrolatum in a 60:40 proportion could be used as an effective technique to measure differences in root penetration ability of common bean genotypes.

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