CHAPTER FOUR

CHARACTERIZATION OF ROOT TRAITS IN RELATION TO SEED YIELD OF COMMON BEAN LINES GROWN UNDER WELL-WATERED AND DROUGHT CONDITIONS IN THE FIELD

Bean inbred line selection for the field trial conducted was done in consultation with my co-supervisor at CIAT. The Ukulima Root Biology Center (URBC), a CIAT partner, hosted me to conduct the field experiment. The research group at URBC further helped me in gaining practical knowledge to phenotype roots. I was responsible for planning and execution of the study. For root image analysis using the Winrhizo software, I was first trained in Ethiopia. The carbon isotope discrimination and natural abundance of $\delta^{15}N$ analysis for all field experiment was done at the University of Cape Town.

4.1 Abstract

The physiological basis of differences in field performance of nine common bean lines was determined at the Ukulima Root Biology Center, Limpopo Province, South Africa. Root morphology traits (root length, surface area, volume and average diameter) as well as root architecture traits (branching density, whorl angles) of the tap, basal and adventitious bean roots were measured under drought and well-watered conditions in the field. Also, chlorophyll content of leaves, plant biomass and seed yield were determined under both well-watered and drought conditions. Drought stress affected both morphological and architectural root traits, however three bean lines (BAT 477, BT_34-1-1 and PAN 185) performed better under stress. The superior performance of these three lines was due to higher canopy biomass and seed yield when compared to all other lines. Effective use of water through enhanced lateral root development and maintaining the water status of the plant were very likely the key factors for enhanced productivity under water deficit. Results obtained further showed that root length, area and volume as well as first whorl angle, basal root number and adventitious root branching density were significantly related to seed yield.

4.2 Introduction

Common bean is mostly grown under rainfed conditions in the tropics. Drought severely affects carbon and nitrogen fixation decreasing plant dry mass and plant productivity (Fenta et al., 2011). Therefore, it is important to develop bean varieties with better water use efficiency. For drought and also nutrient stress adaptation, root architecture and morphology are important traits (Beebe et al., 2006; Lynch, 2007; Zhao et al., 2004). However, little information is currently available to use root architecture or morphology as parameters to evaluate bean performance under drought. Sponchiado et al. (1989) found significant differences in the rooting ability among bean lines with BAT 477 forming deep roots under drought. Enhanced root mass is often considered to be related to reduced yield. However, White and Castillo (1991) outlined that the ability to produce a high root mass under drought in common bean was associated with higher harvestable yield. Further in chickpea, root length density correlated with drought tolerance and higher yield (Kashiwagi et al., 2006). Previous research has also shown that a deep and dense root system in common bean (Kobata et al., 1996) or high root mass (Fenta et al., 2011; Mohamed et al., 2002) correlates with effective water use under drought conditions. However, a detailed study to investigate root characteristics as morphological markers for drought tolerance in common bean has so far not been done.

The objective of this chapter was therefore to test if root architecture and morphology traits for drought tolerance can be used to identify superior performing bean lines for drought tolerance under field conditions. A further objective was to evaluate if root architecture and morphology traits directly relate to seed yield. For this, root architecture and morphology traits were

measured in different nitrogen-fixing and non-fixing bean lines and finally related to seed yield. Results obtained show both morphological and architectural root traits were significantly affected by drought with bean lines BAT 477, BT_34-1-1 and PAN 185 performing superior under drought. Further, root length, area and volume as well as 1st whorl angle, basal root number and adventitious root branching density were significantly related to seed yield under drought.

4.3 Materials and methods

4.3.1 Experimental site

Experiments were conducted during the 2010 cropping season (February to May) at the hosting institute of Ukulima Root Biology Center (URBC), operated by Natural Conservation Thrust, Limpopo Province, South Africa (24^{0}32.002'S, 28^{0}07.427'E and 1237m above sea level). The area had the following climatic conditions: average total annual precipitation 623 mm, average maximum/minimum temperature 26-28/13-17^{0}C during the growing season with a 1500-1800 mmol m^{-2} s^{-1} average PAR (data were generated using the MarkSim™ simulation software developed by the International Centre for Tropical Agriculture (CIAT) using 100 year climatic data).

The soil texture of the field was sandy according to the soil classification (USDA, 2011). The soil can be described when dry as loose with single grains that feel coarse and that fall apart when released and when moist, forming cast and when squeezed, the cast crumbles on touch and

does not form a ribbon. Prior to experiments, a soil analysis for both macro- and micro-nutrients was conducted by the Alpha Agric PLC soil analysis laboratory, Nylstroom, South Africa. Nutrient analysis revealed available P 18 mg/kg, K 50 mg/kg, Na 12 mg/kg, Ca 196 mg/kg, Mg 57 mg/kg and Fe 4.62 mg/kg, Mn 2.37 mg/kg, Cu 0.15 mg/kg, Zn 0.85 mg/kg by extracting soil sample in diethylene triamine pentaacetic acid (DTPA) and 1.63 cation exchange capacity (CEC) and a pH (in KCl) of 5.82. Based on the recommendation made by the laboratory 4kg/ha boron, 1 kg/ha zinc sulfate and 25 kg/ha potassium sulfate were applied to overcome nutrient limitations in the soil.

4.3.2 Plant material

Overall, nine common beans (*Phaseolus vulgaris* L.) lines were used in this field experiment. Four common bean inbred lines (BT _6-1-1, BT _34-1-1, BT _51-1-1 and BT _147-3), two parental lines (DOR 364 and BAT 477), and two mutant lines that have lost the capacity to nodulate (DOR 364-NN and BAT 477-NN) acquired from CIAT as well as one commercial nitrogen-fixing cultivar widely grown in South Africa (PAN 185) were used. Moreover, three soybean cultivars were tested with these bean lines for comparative analyses. Overall, the experiment was conducted using twelve genotypes as treatments.

4.3.3 Pest control

Before land preparation, a post-emergence, non-selective herbicide Agroquat (Syngenta crop protection, Inc.) and Roundup (Monsanto Plc) at 3 L/ha were applied to kill all above-ground

green tissue of actively growing plants on the field. The land was prepared by plowing and row-making using a tractor with mounted farming implements. Ahead of planting pre-emergence herbicides Unimoc (Meridian Agrochemical Company (Pty) Ltd) EC 800 ml/ha and Imazethaphyr (American Cyanamid Co., US) 400 ml/ha were applied to control both grass and broadleaf weeds. Frequent hand-weeding was also done upon demand. To prevent nematode infestation, immediately after planting and after a month of planting, a nematicide Oxamyl (SinoHarvest Agrochemical Manufacturer, China) (3 L/ha) was applied.

4.3.4 Experimental design

The experimental design was a randomized complete block with two treatments (Appendix 3). Plants were grown in one treatment under adequate water supply where plants were irrigated at a regular interval to keep the soil moisture status near field capacity. The second treatment received a limited water supply and water stress was initiated one month after planting. During the first four weeks of growth, plants were watered regularly (8 mm/day) using pivot sprinkler irrigation to maintain optimum growth conditions. After one month, the water-stressed block was subjected to water deficit by withholding irrigation. However, the trial was exposed to three days of rain at 7^{th}, 19^{th} and 26^{th} days after commencement of drought with 14, 9 and 11 mm (a total of 34 mm rain) respectively. The interference of the rain was not affected the drought experiment, as it was planned to apply once per week irrigation for drought plots. Drought stress lasted for one month, after that both treatments received rain again.

Plants for each treatment were planted in five rows with spacing of 75 cm x 10 cm between rows and plants, respectively. Row length was 4 m with a single plot size of 12 m^2. Distance between rows was deliberately increased to facilitate root sampling at harvest and allowing movement of farm implements. Four rows were used for data collection and the outside row was used as a border. Three replicates were used in each treatment (Appendix 3). Between plots, 75 cm space was left and 1.2 m between replication and 1m border. The two water regimes were separated by 4 m space. The experiment covered a total of 1709.2 m^2 area (Appendix 3). One seed per hole was planted using a jab planter which allowed to plant with a uniform 5 cm depth.

4.3.5 Measured parameters

4.3.5.1 Soil moisture content

Volumetric water content was measured to evaluate the water status of the soil at the initiation of the drought treatment, and every five days for another four times during crop development. Soil sampling was conducted by taking a soil core using a steel corer lined with a plastic tube (60 cm length and 42 mm diameter) acquired from Giddings Machine Company Inc. Four samples per replication (twelve samples) were taken from each irrigation regime. After determining the mass of wet soil, the soil was oven-dried for 48 hrs at 105^0C. Finally, the volumetric water content (θ_v) was calculated using the following formula (Brady and Weil, 2008).

$$\theta_v = \frac{[\text{wet soil weight} - \text{dry soil weight}]}{[\text{water density} * \text{volume of soil}]} * 100$$

4.3.5.2 Chlorophyll content

Three plants of each variety per plot (nine plants per water regime treatment) were sampled at the beginning and at the end of the drought stress treatment using the central leaflet with same age of the 3th and 4th trifoliate leaf. Chlorophyll content of leaves was measured using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing, Inc., Japan) and chlorophyll content was determined non-destructively by taking the average of three individual SPAD chlorophyll meter readings (SCMR).

4.3.5.3 Root architecture

Phenotyping for root architecture for main root types (Figure 4.1) was carried out at flowering stage of plants by taking six representative individual plants per plot for each water regime. For determining root architecture, roots were carefully harvested by applying a "Shovelomics"(Lynch, 2011; Trachsel et al., 2011) technique (Figure 4.2) using a shovel and gently washing the root by water. Tap root width (thickness) was determined by measuring the diameter of the tap root 2 cm away from the root origin. The branching density was determined by counting the lateral roots on a 2 cm root segment from the tap root. Number of whorls was measured by counting. The whorl angle was determined by displaying the root on 180^0 protractor sketched board (similar to the root in the soil) where the stem is at 0^0 (Figure 4.3). The angles on both sides of the stem were measured and the average of measurements was determined. The total number of basal roots was recorded by counting from the whorls. Basal and adventitious root diameter (thickness) was calculated by selecting representative basal/adventitious roots or by taking the average of the diameter of two or three basal/ adventitious roots 2 cm away from

the root origin. Branching density was determined by taking a representative area from the basal/adventitious root and counting the healthy lateral roots emerging within 2 cm root segment for three randomly selected basal/adventurous roots. All diameters (thickness) were measured with an Electronic Digital Caliper 5HA 1890 Model (Omni-Tech electronic Co. Limited, China).

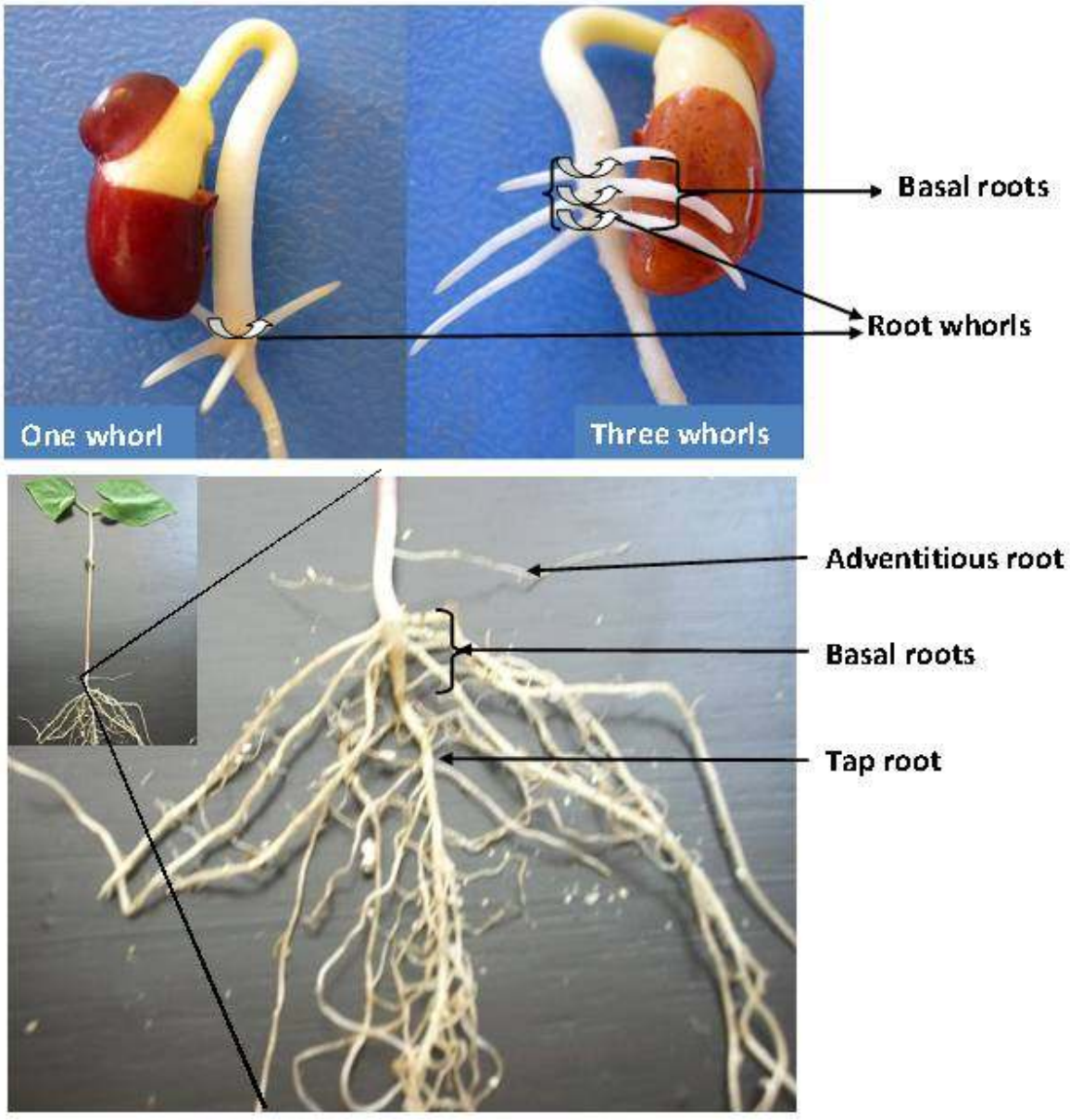

Figure 4.1 Schematic representation of common bean root system architecture with root whorl and main root types.

Figure 4.2 Excavation of the plants using the "Shovelomics" technique which involves digging the plant carefully with two shovels at a time in two directions about 20 cm away from each side without disturbing the root system with the soil.

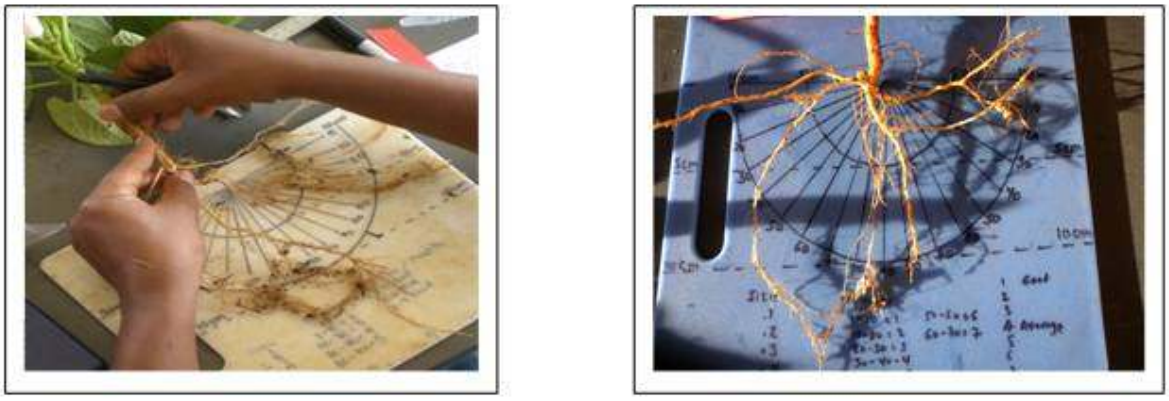

Figure 4.3 Whorl angle measurements by displaying the root on an 180^0 protractor sketched board.

4.3.5.4 Root morphology analysis

4.3.5.4.1 Soil coring

Soil coring was carried out for quantifying root distribution across soil depth. Three soil samples were taken for each plot under well-watered and drought conditions. A total of 162 soil cores were sampled as described for soil moisture content. The soil core samples were collected at a point mid-way between the two plants (Figure 4.4).

4.3.5.4.2 Root washing and scanning

After coring, the soil core was cut into 10 cm pieces (up to 40 cm soil depth) with fifth cut of 20 cm (40 to 60 cm soil depth). Each segment was washed using a 2 mm size mesh. Separated roots were kept in plastic vials with 25% ethanol (Figure 4.4). Ethanol was diluted with water purified with a Milli-Q® Integral system **(**Millipore Corporation, Billerica, MA, U.S.A, 2008). The washed and preserved roots were scanned using the root scanner Epson Perfection V 700 Photo /V 750 Pro (Seiko Epson Corporation 2005) (Figure 4.4). Scanned images were analyzed using the winRHIZO 2008a software as an image analysis system specially designed for root morphology measurements (Regent Instruments Canada Inc., Canada) in Ethiopia. Using this software, root morphological data of root length, average diameter, total area and volume were determined.

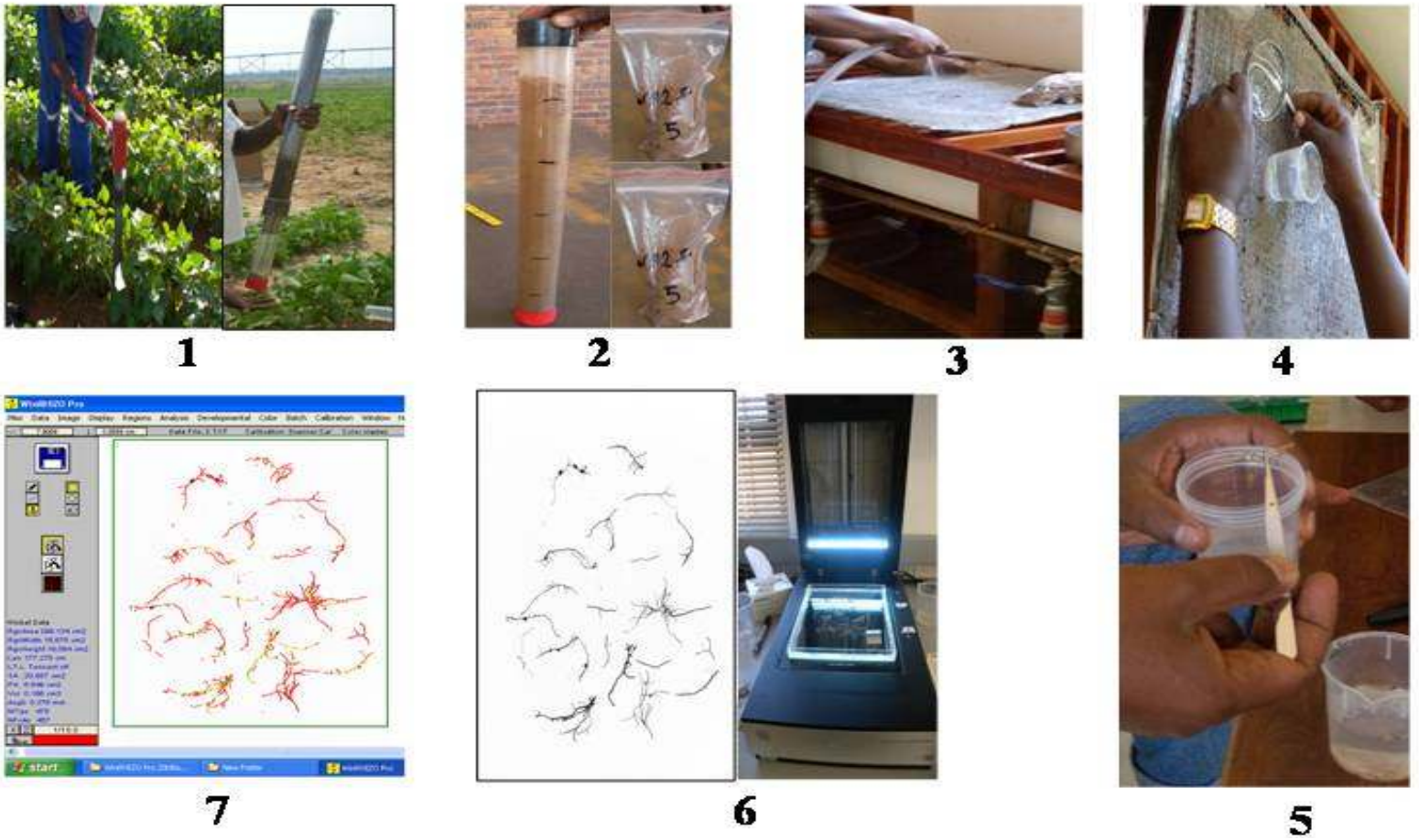

Figure 4.4 Steps involved in root morphology analysis, step 1 (soil coring), step 2 (cutting into pieces), step 3 (washing), step 4 (separating root from foreign materials), step 5 (preserving the root in 25% ethanol), step 6 (scanning root using root scanner Epson Perfection) and step 7 (analyzing the scanned root images using Winrhizo software)

4.3.5.5 Biomass partitioning and seed yield measurement

Whole above ground plant samples of six representative individual plants per plot for each water regime were harvested at flowering and at mid-pod filling stage. The vegetative parts were carefully separated into leaves, stems and pods (at mid-pod filling stage). Dry mass were determined by drying plant material in an oven (TERM-O-MAT LABOTEC, South Africa) at 60^0C for 48 hrs. For determining seed yield from each plot per treatment, two rows of 3 m length (2.25 m^2 area) were used, disregarding a border (0.5 m) on both extremes of the rows. For calculating the harvested plot area, harvested plants were counted and used to calculate the exact area according to the number of the plants harvested to standardize the plot area using the formula:

$$\text{Seed yield } (2.25\ m^2) = \frac{\text{Measured seed yield x 2.25}}{\text{Calculated harvested area}}$$

Grain yield was determined after measuring and adjusting the seed moisture content at 10% using the method of oven-drying moisture content (MC) measurement applying the following formula.

$$\text{Adjusted seed yield } (g) = \frac{\text{Seed yield } (2.25\ m^2) \text{ x } 10}{\text{Measured MC}}$$

Productivity of common bean lines/ha was calculated using the equation:

$$\text{Yield } (kg/\ ha) = \frac{\text{Adjusted seed yield } (g) \text{ x } 10}{2.25}$$

For determining the biomass partitioning ability of plants of various lines, all plants from one row (3 m length) were counted and harvested independently and then the pod wall and seed were separated carefully by splitting by hand. Samples were dried in an oven at 60^0C for 2 days and the dry mass was determined. Data were used to calculate the pod harvest index (PHI) using the following formula as it has been also applied before by CIAT for varietal evaluation (Beebe et al., 2010).

$$\text{PHI} = \frac{\text{Seed biomass dry weight at harvest}}{\text{Pod biomass dry wight at harvest}} \text{ x } 100$$

4.3.6 Statistical analysis

Data were analyzed using the JMP® 9.0 statistical package (SAS Institute Inc., Cary, NC, USA). Analysis of variance was used to determine significance and LSmeans student's t-test was used to compare bean lines for measured traits. Multivariate Pearson's correlation analysis was used for determining the relationship (correlation) between measured traits.

4.4 Results

4.4.1 Soil moisture content and chlorophyll content

Before exposure to drought conditions, the volumetric soil water content was determined to be about 14% for both the well-watered and drought blocks (Figure 4.5A). This was comparable to the field capacity for sandy soil previously reported by Brady (2008, Figure 4.5B). Almost constant volumetric water content were found under well-watered conditions, while a progressive decrease of the soil water content was observed in the drought treatment. The soil water content decreased to 7.4%±0.55 after 4 weeks of drought. This was a 45% reduction in the volumetric water content in the drought plots when compared to the well-watered plots. The drought treatment was only effective for 4 weeks because the experimental farm received rain and the soil water status immediately rose.

Drought significantly reduced leaf chlorophyll content by about 19.34% for the two non-fixing lines DOR 364-NN and BAT 477-NN and about 10% for all N-fixing lines after 4 weeks of stress when compared to well-watered lines with BT_51-1-1 having the lowest chlorophyll content among N-fixing lines (Figure 4.6).

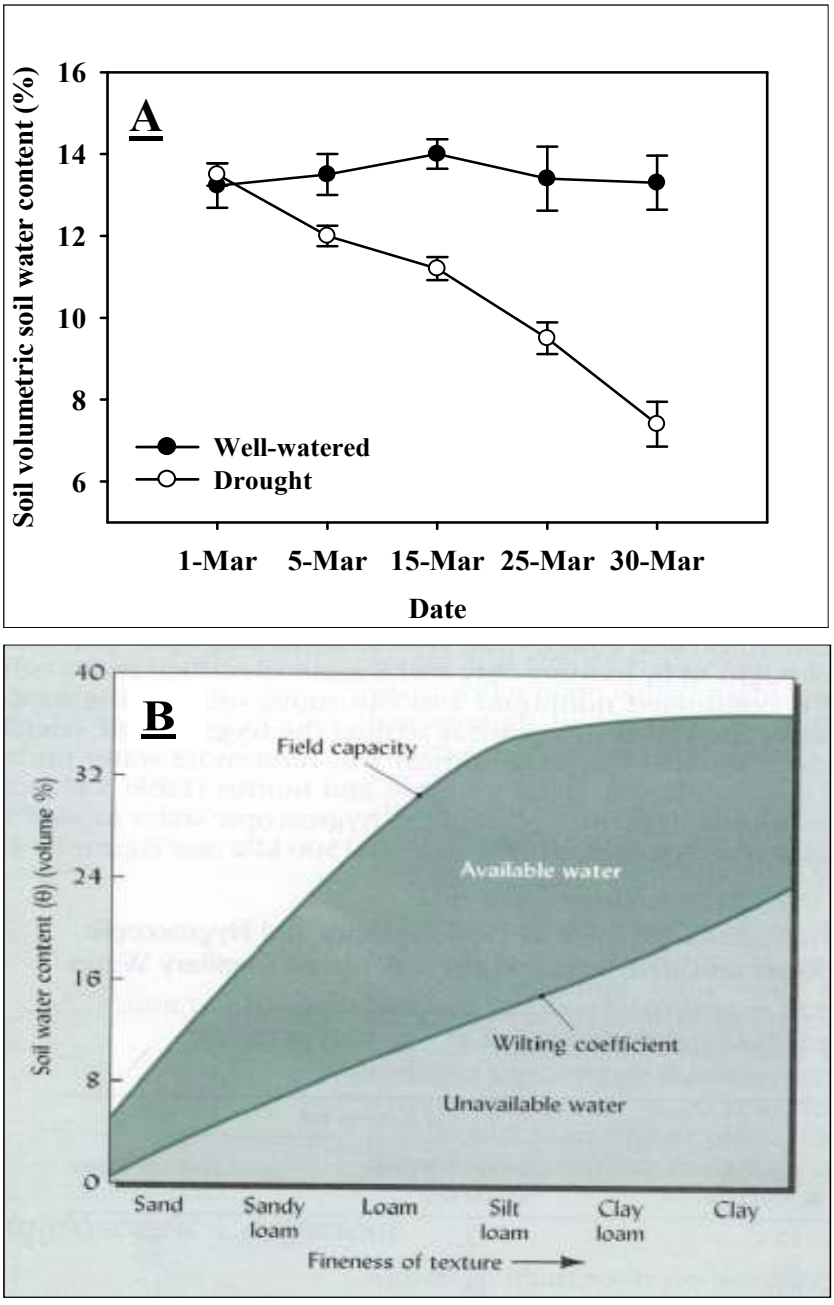

Figure 4.5: (A) Soil volumetric water content values (%) for drought and well-watered blocks on which plants of nine common bean lines were grown. Values represent the mean ± SEM of four soil samples per replication (twelve samples) for each irrigation regime. (B) Soil volumetric water related to soil texture class for visualization of water status of soil (Brady and Weil, 2008).

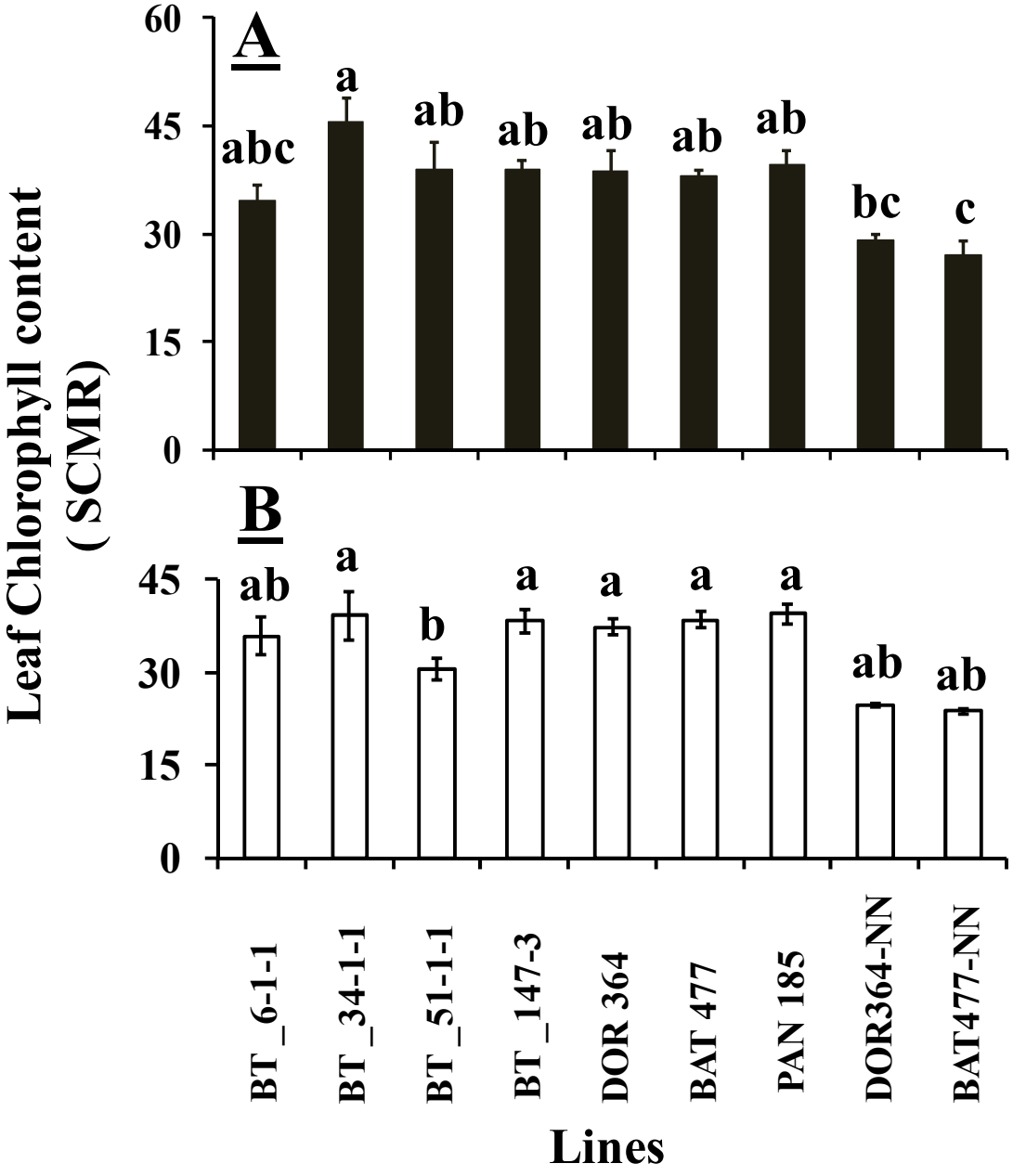

Figure 4.6 Effect of water deprivation on leaf chlorophyll content (SCMR) of nine bean lines measured after three weeks of drought stress at the water-limited treatment. Values represent the mean ± SEM of three plant samples of each variety per plot (nine replicates per treatment) (A) well-watered and (B) drought. Different letter on bar denote significant difference ($P<0.05$).

4.4.2 Root morphology and architecture

When analysis of variance was carried out for root morphology and architectural traits for two ways ANOVA,, lines X water treatment interaction was not significantly different ($P>0.05$) (data not shown). Thus, the main effects were evaluated. Accordingly, the analysis of variance for bean lines for all root morphology traits, were significantly different ($P<0.05$) under drought condition except for average root diameter (Appendix 4),, nevertheless, none-significant differences ($P>0.05$) were found for all root morphology traits when plants of various lines were grown under well-watered conditions (Appendix 4).

Relative to the non-stressed treatments, common bean lines subjected to drought responded by increasing the values of root morphological parameters (root area, volume and length) between 15-20% when compared to well-watered conditions (data not shown). Among the tested lines, BT_34-1-1, BAT 477 and commercial cultivar PAN 185 had higher values for root morphology traits when compared to all other lines. The values of the two non-nodulating bean lines (DOR 364-NN and BAT 477-NN) were lower than the best performer lines by 50% for all root morphological traits except for root diameter relative to nodulating lines (Table 4.1).

Regardless of water regime used the first and second whorl angles of roots were significantly different ($P<0.05$) among lines (Appendix 5). Only tap-root branching density and also basal root number and branching density were significantly ($P<0.05$) different between lines under drought (Appendix 5). Further, adventitious root width and branching density was significantly ($P<0.05$)

different between lines under both water regimes and also adventitious root number but only under well-watered conditions (Appendix 5).

Under both water regimes, the number of whorls was between 1.6 and 2.13. Irrespective of the water treatment,, the arrangement of the first whorl angle was categorized into three groups: first group with a 1st whorl angle (42^0-49^0) consisted of PAN 185, BT_34-1-1 and BAT 477, the second group with 36^0-38^0 (BT_6-1-1 and BT_51-1-1) and the third group (31^0-35^0) the remaining lines. For the second whorl angle, except for BT_147-3, BT_6-1-1, and BAT 477-NN with a 2nd whorl angle of 33^0-37^0, all other lines had a similar root 2nd whorl angle of 40^0-50^0 (Table 4.2). Further, BT_6-1-1 had the highest tap root branching density under drought followed by BT_34-1-1, BAT 477, BT_51-1-1 and DOR 364 (Table 4.2). However, for other root architectural traits (basal root number and branching density of well as adventitious root branching density) BT_34-1-1 and BAT 477 were shown consistently higher performance than other bean lines (Table 4.3).

Table 4.1 Differences in root morphology traits of nine bean lines grown under drought treatment. The root image was taken by a root scanner and analysis was made by using the winRHIZO 2008a software after 4 weeks of drought

Lines	Root length (cm)	Surface area (cm^2)	Root volume (cm^3)	Root tips	Diameter (mm)
N-fixing lines					
BT _6-1-1	78.67±15.44b	10.37±1.95bc	0.103±0.02bcd	285.30±46.5bc	0.44±0.02
BT _34-1-1	93.56±16.9ab	11.83±2.04ab	0.132±0.02ab	327.6±60.6abc	0.43±0.03
BT _51-1-1	61.71±13.55b	6.79±1.61bc	0.117±0.02bc	204.28±35.2c	0.51±0.04
BT _147-3	79.36±20.26b	10.25±2.18bc	0.099±0.02bcd	318.19±55.3abc	0.40±0.02
DOR 364	57.25±14.27b	7.01±1.73bc	0.075±0.02cd	235.41±41.2bc	0.38±0.02
BAT 477	132.15±23.47a	15.73±2.54a	0.161±0.03a	418.40±63.1a	0.41±0.02
PAN 185	83.86±13.5b	11.18±1.91a	0.122±0.02ab	342.76±47.0ab	0.45±0.02
Non-fixing lines					
DOR 364-NN	55.91±11.29b	6.28±1.33c	0.064±0.01d	206.81±36.2c	0.44±0.03
BAT 477-NN	52.23±12.17b	7.35±1.35bc	0.073±0.01cd	237.11±34.8bc	0.41±0.02
Significance	*	*	**	*	ns

Significance level was determined using ANOVA ($^{**}P<0.001$, $^{*}P<0.05$, and ns $P>0.05$) and difference between treatment means was determined using the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different. The result is the mean ± SEM of four replicates for each treatment acquired soil core up to 60 cm soil depth.

Table 4.2 The performance of nine bean lines using mean separation for root architecture traits in a drought treatment.

Lines	Whorl numbers	1st Whorl angle	2nd Whorl angle	Tap root width (mm)	Tap root branching density
N-fixing					
BT _6-1-1	2.00±0.0	38.6±2.6bcd	34.1±2.3c	1.63±0.18	10.76±0.88a
BT _34-1-1	1.87±0.09	45.3±3.1ab	50.7±2.3a	1.36±0.16	8.57±0.92ab
BT _51-1-1	2.00±0.00	36.2±4.7bcd	43.1±5.7abc	1.88±0.24	7.60±0.5bc
BT _147-3	1.87±0.09	31.7±3.6d	37.7±3.7bc	1.63±0.28	5.73±0.9cd
DOR 364	2.00±0.00	36.7±3.0bcd	47.33±2.6ab	1.71±0.23	8.07±0.85bc
BAT 477	2.00±0.09	49.7±2.6a	43.31±3.5abc	1.78±0.22	8.73±0.61ab
PAN 185	1.93±0.20	42.3±2.6abc	40.0±3.3abc	1.79±0.25	6.6±0.54bcd
Non-fixing					
DOR 364-NN	1.71±0.12	35.7±2.4cd	43.0±3.2abc	1.54±0.19	7.4±1.29bcd
BAT 477-NN	1.80±0.14	35.0±4.0cd	37.3±2.2bc	1.04±0.16	5.20±0.54d
Significance	ns	**	*	ns	**

Significance level was determined using ANOVA (**P<0.001,, *P<0.05, and ns P>0.05) and difference between treatment means was determined using the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different. The result is the mean ± SEM of six representative plants per plot exposed to 4 weeks of drought.

Table 4.3 The performance of nine bean lines using mean separation for root architecture traits in a drought treatment.

Lines	Basal root number	Basal root width (mm)	Basal root branching density	Adv. Root number	Adv. root width (mm)	Adv. root branching density
N-fixing						
BT _6-1-1	6.14±0.22bc	1.04±0.09	8.00±1.21ab	8.6±0.9	0.51±0.05bcd	9.67±0.9a
BT _34-1-1	7.27±0.37ab	0.89±0.13	9.71±0.91a	11.7±1.8	0.46±0.08bcd	7.07±1.0ab
BT _51-1-1	6.8±0.3abc	1.23±0.12	7.47±0.5ab	8.1±1.3	0.45±0.9bcd	4.5±0.9bcde
BT _147-3	5.93±0.39c	1.04±0.11	7.07±1.11ab	7.6±0.8	0.38±0.06cd	5.6±0.8bcd
DOR 364	6.33±0.41bc	0.92±0.12	6.60±0.74b	7.3±1.0	0.30±0.1d	2.79±0.7e
BAT 477	7.67±0.41a	0.77±0.1	9.53±1.25a	9.6±0.8	0.65±0.04ab	5.7±0.9bcd
PAN 185	6.86±0.67abc	1.15±0.14	8.0±0.47ab	8.4±1.2	0.80±0.06a	6.0±0.9bc
Non-fixing						
DOR 364-NN	5.71±0.44c	1.22±0.21	6.86±0.99b	5.5±1.5	0.38±0.05cd	3.43±0.6de
BAT 477-NN	6.33±0.46bc	0.92±0.13	5.40±0.61b	6.6±b1.6	0.55±0.11bc	3.9±0.9cde
Significance	*	ns	*	ns	**	**

Note: Adv= adventitious roots

Significance level was determined using ANOVA ($^{**}P<0.001$, $^{*}P<0.05$ and ns $P>0.05$) and difference between treatment means was determined using the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different. The result is the mean ± SEM of six representative plants per plot under drought growth condition after the exposure for one month moisture stress.

4.4.3 Days to maturity, biomass, and yield

Under well-watered conditions bean lines matured in 86-102 days (data not shown) and 81-96 days under drought (Figure 4.7). The rather small difference between the two conditions was possible due to rainfall occurring during the field experiment. The earliest maturing bean line was BT _6-1-1 (81 days) and PAN 185 the latest (96 days) (Figure 4.7).

Marked influences of genotype and water treatment on biomass and seed yield were ascertained by the two way analysis of variance (Appendix 6). Accordingly, dry total shoot mass at flowering stage, shoot dry mass (leaf, pod and total) at mid pod filling stag as well as seed yield were revealed a significant influence on bean lines on their performance response to water treatment (drought) as indicated by a significant interaction of lines X water treatment (Appendix 6). Furthermore, the main effect of one way ANOVA for bean lines on above ground dry biomass both at flowering and mid-pod filling stage revealed significant differences under well-watered and drought conditions (Tables 4.4 and 4.5).Under well-watered conditions, the two non-fixing bean lines DOR 364-NN and BAT 477 NN and nitrogen-fixing line BT_147-3 produced significantly less ($P<0.05$) regarding biomass and seed yield than all other lines at flowering and mid-pod filling stage (Table 4.4). Further, PAN 185 accumulated significantly higher ($P<0.05$) total shoot and leaf biomass than all other lines, followed by BAT 477, at both time points under drought (Table 4.5). However, highest pod dry mass under drought was found for line BT _51-1-1 followed by line BAT 477 (Table 4.5) and highest total biomass was observed for lines, BAT 477, PAN 185, and BT_51-1-1 (Table 4.5).

Bean lines exhibiting higher PHI and biomass (except for BAT 477 at flowering stage) under well-watered condition also had significantly ($P<0.05$) higher seed yield than all other lines. Under well-watered conditions, all N-fixing lines had a higher seed yield (2.6-2.8 t of grain yield/ ha) when compared to non-fixing lines (1.4 t/ha) (Figure 4.8A). Under drought, significant differences were found among tested bean lines for seed yield (Figure 4.8B). The decline in seed yield for the tested bean lines were ranged from 23 to 50%. The highest seed yield reduction (>40%) was observed for DOR 364, BT_147-3 and Bt_6-1-1, however, the lowest reduction of yield due to drought stress was observed for earliest bean line BT_34-1-1 and better performing cultivar PAN 185 and lines BAT 477 (Figure 4.9). PAN 185, BT_34-1-1 and BAT 477 had relatively higher PHI (Table 4.4) under drought and also had higher harvestable seed yield. Further, a significant ($P<0.05$) relationship was found between seed yield and root morphology traits (root length, area and volume) (Table 4.6) ($P<0.05$). Also a positive significant ($P<0.05$) relationship between seed yield and root architecture traits was found for 1st root whorl angle, basal root number and adventitious root branching density (Table 4.6). However, no significant ($P>0.05$) relation was found between PHI and measured root traits (data not shown).

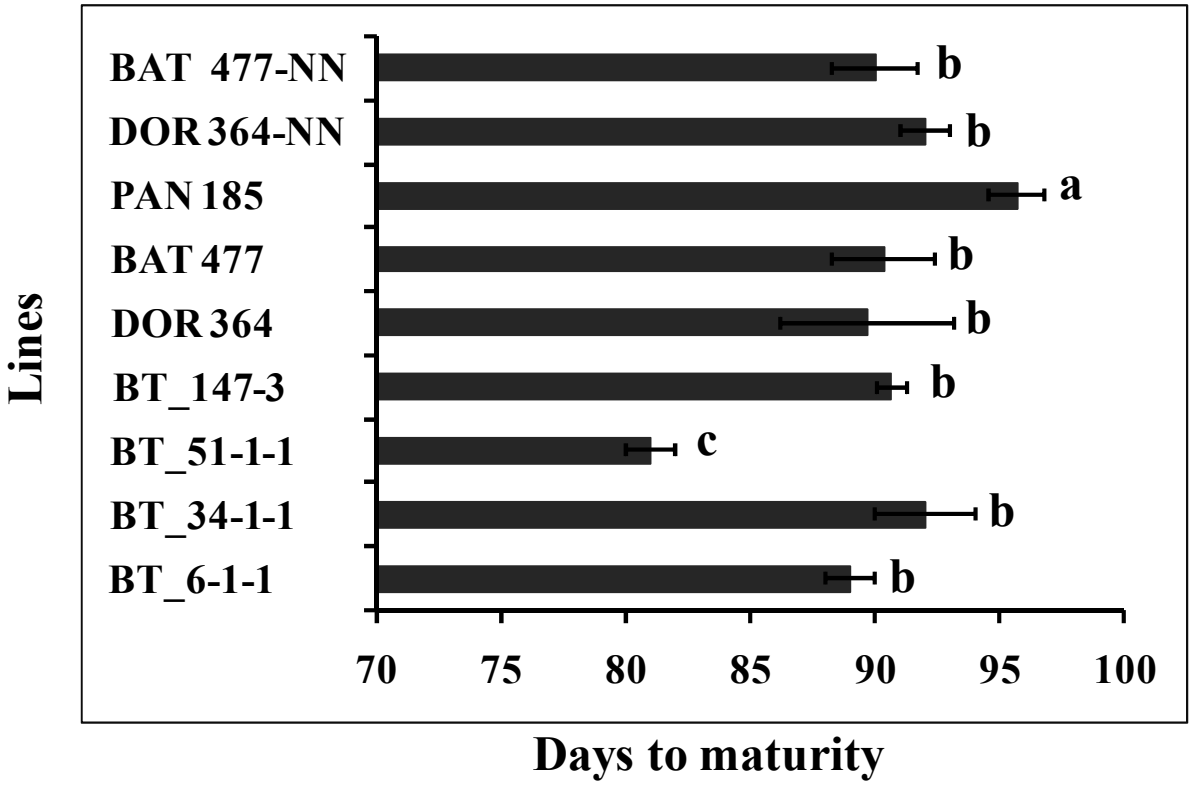

Figure 4.7 Days to maturity of nine bean lines grown under drought conditions. Bars represent the mean ± SEM of each of three plots of each bean lines. Treatment means was determined using the LSmeans Student's t-test and different letter on bar denote significant difference ($P<0.05$).

Table 4.4 Performance of nine bean lines for biomass at flowering and at mid pod filling stage and pod harvest index in the well-watered treatment.

Bean Lines	Total dry mass at Fl (g)	Leaf dry mass at MPF (g)	Pod dry mass at MPF (g)	Total mass at MPF (g)	PHI
N-fixing					
BT_6-1-1	11.00±0.28bc	26.30±0.7bc	9.48±0.68bc	60.99±1.69ab	69.79±0.9a
BT_34-1-1	11.23±0.45b	27.31±0.49ab	9.67±0.69bc	60.39±1.4ab	66.29±1.2bc
BT_51-1-1	11.51±0.14ab	27.86±0.95ab	11.73±0.15a	61.89±1.0ab	68.79±0.72ab
BT_147-3	8.51±0.49d	24.26±0.58cd	9.04±0.57bc	57.14±2.01cd	65.22±0.78c
DOR 364	11.45±0.31ab	27.71±1.32ab	10.57±0.56ab	63.72±2.73ab	69.45±0.52ab
BAT 477	11.20±0.3b	29.33±0.52a	11.81±0.52a	63.26±1.48ab	67.98±1.95abc
PAN 185	12.33±0.32a	28.55±0.71a	11.55±0.62a	65.06±1.48a	68.34±0.71abc
Non-fixing					
DOR 364-NN	9.99±0.56c	23.23±0.73d	6.99±0.67cd	55.23±1.32d	60.63±0.59d
BAT 477-NN	8.84±0.23d	24.04±0.53d	8.29±0.52d	55.01±0.74d	57.95±1.6d
Significance	**	**	**	**	**

Fl = flowering, MPE = mid pod filling stage

PHI = pod harvest index

Data represent the mean ± SEM of six representative individual plants per plot (biomass at flowering and MPF) and three replications for each bean lines (PHI) under water-limited growth conditions. Different letter within a column denote significant difference ($P<0.05$).

Table 4.5 Performance of nine bean lines for biomass at flowering and mid pod filling stage, and pod harvest index in the drought treatment.

Bean Lines	Total dry mass at Fl (g)	Leaf dry mass at MPF (g)	Pod dry mass at MPF (g)	Total mass at MPF (g)	PHI
N-fixing					
BT_6-1-1	7.41±0.3c	23.58±0.40cd	7.49±0.8b	54.7±1.44bcd	66.60±0.72abc
BT_34-1-1	9.28±0.19b	24.23±0.52bc	7.93±0.46b	56.40±0.61bc	63.54±2.94abcd
BT_51-1-1	9.15±0.29b	23.46±0.33cd	11.11±0.5a	57.49±0.41ab	71.09±4.88a
BT_147-3	7.62±0.50c	22.76±0.73cd	7.04±0.4b	52.63±0.54de	57.81±0.96bcd
DOR 364	6.80±0.39cd	22.34±0.47cd	7.45±0.6b	51.90±1.33de	54.05±1.95cd
BAT 477	10.1±0.38ab	25.80±0.54ab	9.93±0.44a	60.66±1.56a	63.85±1.2abcd
PAN 185	10.94±32a	26.30±1.07a	7.79±0.3b	60.48±1.24a	67.12±1.52ab
Non-fixing					
DOR 364-NN	7.85±0.45c	22.28±0.44d	6.88±0.4b	53.35±0.78cde	51.81±4.05d
BAT 477-NN	5.90±0.53d	22.79±0.43cd	7.38±0.59b	51.17±0.75e	51.98±1.28d
Significance	**	**	**	**	**

Fl = flowering

MPF = mid pod filling stage

PHI = pod harvest index

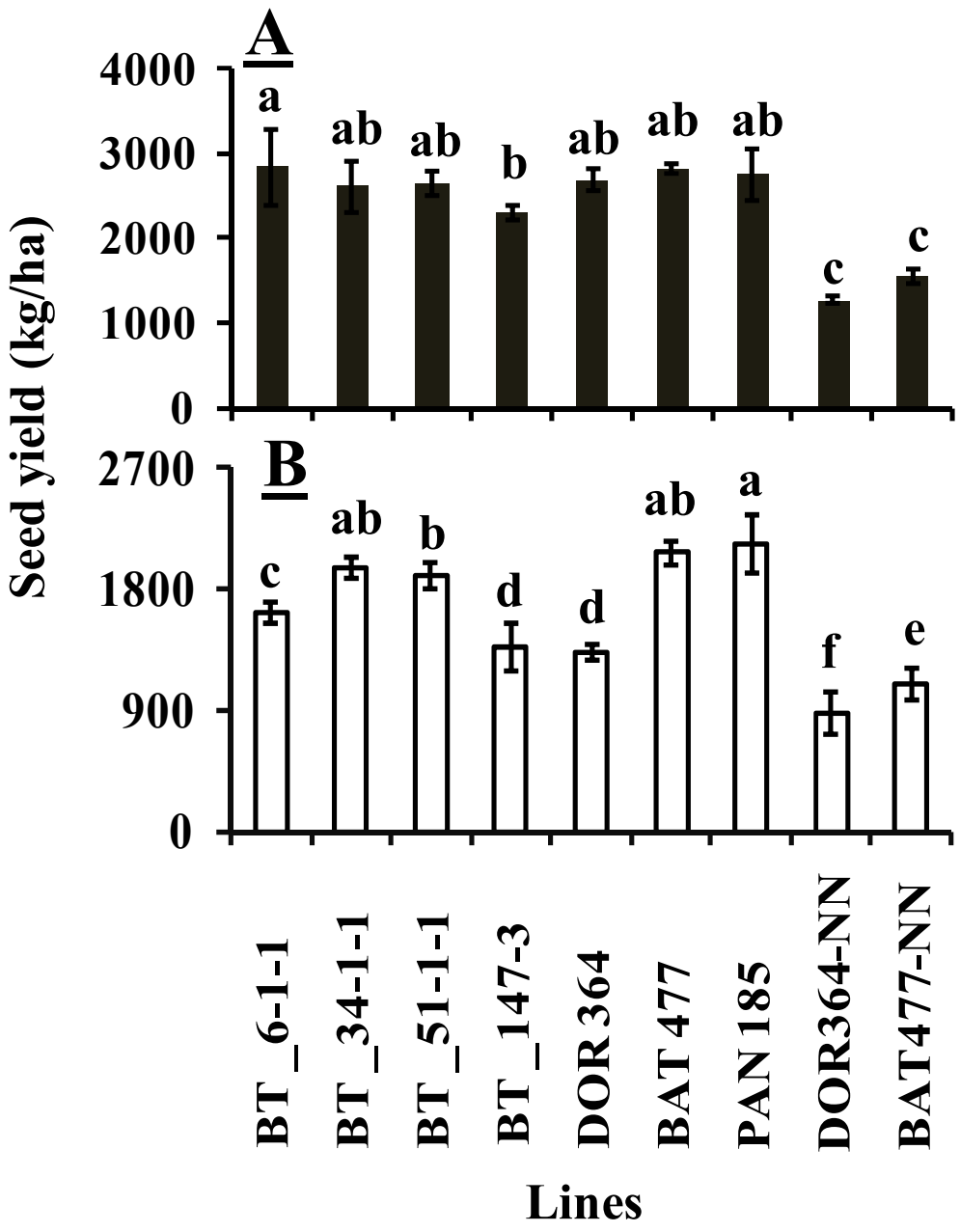

Figure 4.8 Seed yield of nine different common bean lines grown either under well-watered (A, closed bars) or water-limited growth condition (B, open bars). Bars represent the mean ± SEM three replications of two rows of 3 m length for each treatment (2.25 m^2 area) adjusted seed yield at 10% moisture content. Different letter on bar denote significant difference ($P<0.05$).

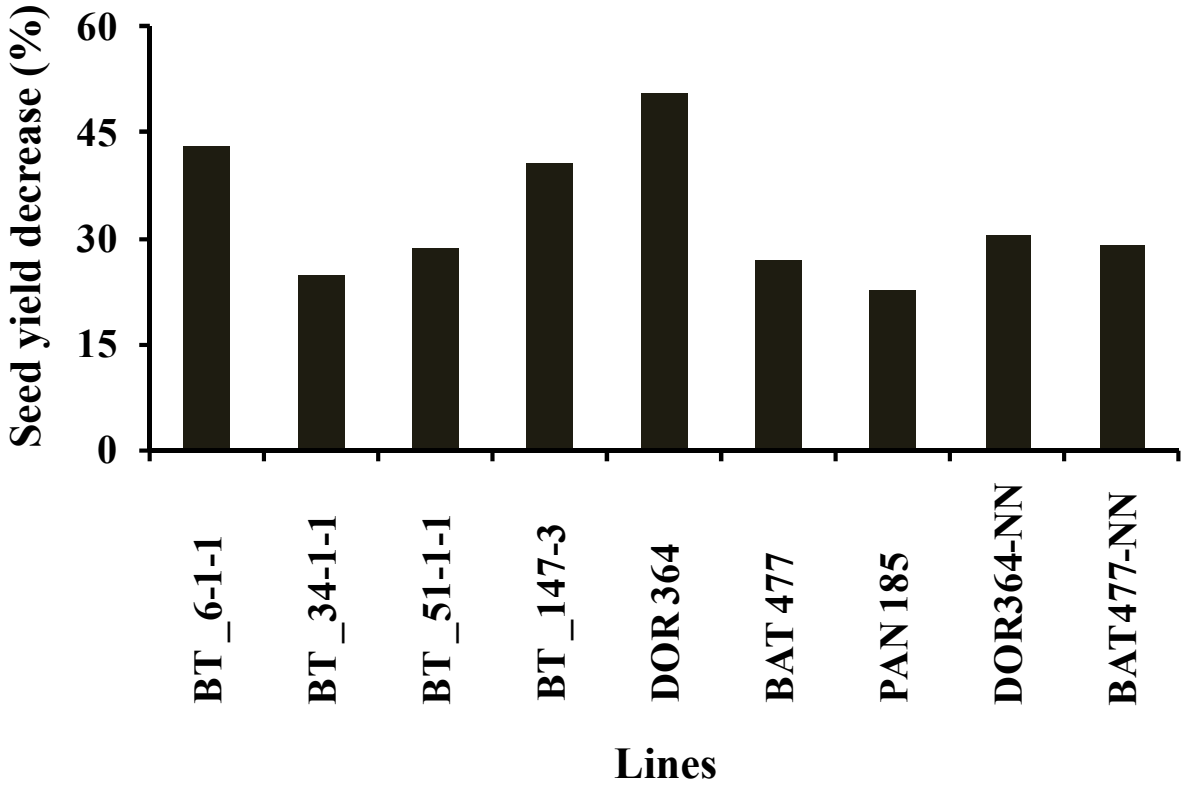

Figure 4.9 Percent decrease of seed yield of nine common bean lines due to water stress. Bars represent the percentage difference of the mean seed yield for three replicates for plants grown under well-watered and water-limited condition in two rows of 3 m length of each treatment. The seed yield was adjusted at 10% moisture content.

Table 4.6 Association of root morphological and architectural traits with seed yield for the pooled data of all bean lines at well-watered and drought growth conditions

Trait	Trait	Well–watered		Drought	
		r	P-value	r	P-value
Seed yield	Root length	0.371	0.3558	0.734	0.0298*
	Root area	0.415	0.4600	0.836	0.0053**
	Root volume	0.520	0.1705	0.876	0.0037**
	1st whorl angle	0.419	0.1561	0.815	0.0096**
	2nd whorl angle	0.354	0.4879	0.193	0.6368
	Basal root number	0.732	0.1942	0.787	0.0171*
	Basal root bran. density	0.6482	0.2242	-0.178	0.9322
	Tap root bran. density	0.386	0.4064	-0.543	0.1875
	Adv. root bran. density	0.468	0.2220	0.503	0.0125*
	Adv. root width	-0.203	0.9322	0.302	0.4600

r = Pearson's correlation coefficient

* indicates the correlation is significantly different ($P<0.05$) and no star indicates the correlation is non-significant ($P>0.05$). Adv = adventitious, bran. = branching.

4.5 Discussion

The objective of this part of the study was to test if root architecture and morphology traits can be used to identify superior performing bean lines for drought tolerance under field conditions. Overall, the study has shown that lines BAT 477, BT_34-1-1, PAN 185 had enhanced root development, high biomass and the highest seed yield among lines under drought and these lines can therefore be considered to perform better under drought. Although line BT_51-1-1 only modestly performed regarding root development and biomass, this line had higher grain yield in addition to earliness in maturity. It may have a drought escaping behavior and might therefore be suited for areas with a short growing season.

The measurement of the chlorophyll content in varietal evaluation has been previously applied as a simple procedure as an indicator for drought tolerance (Minolta, 1990; Smeal and Zhang, 1994). Although SCMR in this study were able to distinguish the chlorophyll content between N-fixing and non-fixing lines, chlorophyll measurement was not sensitive enough to use it to select for drought tolerant lines, as indicated by Munn et al. (2004). This might be partly due to a non-uniform distribution of leaf chlorophyll (Markwell et al., 1995; Uddling et al., 2007). Further, although chlorophyll production might have been affected by drought exposure (Cha-um and Kirdmanee, 2008), this might not necessarily have induced chlorophyll degradation (Chaves et al., 2003).

A further objective was to evaluate if in particular root architecture and morphology traits directly relate to seed yield. For this, traits were measured in different nitrogen-fixing and non-fixing lines and related to seed yield. Root length, area and volume as well as 1st whorl angle,

basal root number and adventitious root branching density were significantly ($P<0.05$) related to seed yield under drought. In this study, superior performing bean lines also had higher shoot biomass (at flowering and mid-pod filling stage) and higher pod harvest index. Furthermore, these lines had also higher grain yield, except for line BT _6-1-1. However, no significant relation between pod harvest index and root traits was found.

The existence of a positive relation of seed yield with root morphological and architectural traits demonstrates the significance of the root for enhanced productivity and potential use of these traits as morphological markers for drought tolerance. Better root system development under drought generally allows extracting soil water from deep soil, which is an important trait for maintaining stomatal conductance and photosynthetic carbon assimilation. Deeper rooting plants providing improved drought tolerance and higher productivity has recently also been reported for rice (Li et al., 2005) and wheat (Reynolds et al., 2007). Passioura (1996) further hypothesized that productivity under drought is the function of the effective use of water (EUW), water use efficiency (WUE) and the ability to convert the photosynthetic assimilate into a harvestable product. Li et al. (2005) and Yadav et al., (1997) also reported that root traits in rice are directly related to drought tolerance. Sinclair and Muchow (2001) further found that in maize enhanced absorption of water due to deep rooting ability is associated with higher productivity. Therefore, in this study better root traits have likely contributed to enhanced water use satisfying the transpiration demand of bean plants and consequently resulting in better shoot biomass and ultimately yield.

Further, root morphology traits, such as root surface area, root volume, length and abundance of root tips, except average root diameter, had in this study a remarkable degree of plasticity due to a changing water status in the field. Since the root is contact with soil, the root is the first site sensor of any change in the soil environment (Osmont et al., 2007). Therefore, with any soil alteration (external stimuli) plants respond with a change in architecture and/morphology which is termed root plasticity (Lynch et al., 2005). Thus, improved performance of a plant depends on how efficient root plasticity is changing in response to a stress. This study clearly showed that bean lines BAT 477, BT_34-1-1, PAN 185, BT_51-1-1, and BT_6-1-1 with superior root morphological architectural traits (root whorl angles, number of basal root and branching density of basal, tap and adventitious roots) were also more drought-tolerant. Additionally, lower reduction of seed yield due to drought stress for bean lines PAN 185, BAT 477, and BT_34-1-1 suggests as these bean lines use their root traits as adaptive strategy to withstand drought stress than other lines. This might be due to a more effective production of hormones to enhance the root system development/plasticity as a response to drought. Changes in root plasticity are due to hormonal changes and auxin plays a major role in root development by controlling the emergence as well as the development of lateral roots (Casimiro et al., 2001; Lucas et al., 2008; Nibau et al., 2008).

Since, measured performance traits for biomasses and seed yield were varied by the moderator variable water stress, the existence of bean lines vs. water treatment interaction for these traits suggests, the severity of the response to these productivity traits differs as a function of the level of water stress. These interactions are a major source of variation for the plant adaptability to water deficit conditions. Furthermore, productivity traits are pertinent for selecting bean lines for

specific water regime combination. For instance parental lines and all inbred N-fixing lines except BT_147-3 can be selected for non-stressed but under water-limited condition BT_34-1-1, BAT 477, and PAN 185 can be selected based on their seed yield as revealed at figure 4.8. Further, the non-significance interaction of the multivariate variance analysis of root traits with water regime also revealed as these traits had a consistent performance across the two growth condition. As a result, a trait which showed consistent performance and also positively associated to seed yield is known to provide a good selection criterion, as long as the genetic diversity exists (Shenkut and Brick, 2003). Hence, measurement of these root traits might be a useful inclusion in bean varietal improvement programs.

In conclusion, this study has shown that root architecture and morphology traits are directly related to drought tolerance in beans. According to Zhao et al. (2004), root angle of soybean was classified in to three, shallow ($<40^0$), intermediate ($40\text{-}60^0$) and deep ($>60^0$) root. Thus, in this experiment BT_34-1-1, PAN 185 and BAT 47 exhibit 40^0-60^0 whorl angles (primary and secondary), therefore, can be grouped under intermediate root architecture. Based on previous studies also, it has been determined that, plants with higher root angle (deeper root) has a capacity to absorb water from deeper soil and perform better under water-limited condition (Singh et al., 2010; Zhao et al., 2004). However, the shallow rooted plants perform better under low phosphorous soil (Lynch and Brown, 2001). Traits, such as root length, area and volume as well as 1^{st} whorl angle, basal root number and adventitious root branching density, significantly related to seed yield under drought and measurement of these traits might be a useful inclusion in bean varietal improvement programs. In particular, measuring root architectural traits is quick, less labor intensive and easy to apply for any bean germplasm screening. Although measurement

of root morphological traits requires a specialized root scanner and software, the technique is also not highly complex and it is easy to handle.

In the next chapter a study on changes in performance traits, such as WUE and symbiotic nitrogen, will be reported and the relation of these traits to root and nodule performance traits investigated.