CHAPTER THREE

PERFORMANCE OF COMMON BEAN UNDER WATER DEFICIT IN A CONTROLLED ENVIRONMENT

3.1   <u>**Abstract**</u>

Common bean is severely affected by drought stress. In this part of the study the effect of drought on plant performance including nodule performance was investigated in six common bean lines that differ in agronomic characteristics. Plants were grown in an environmentally controlled phytotron. Under drought, plants of the various lines tested differed greatly in CO_2 assimilation, stomatal conductance, leaf area, shoot and root mass as well as nodule mass and SNF activity. In drought-stressed plants, leaf water potential and gas exchange were reduced but plants were able to maintain their leaf water status under drought due to better root growth and better CO_2 assimilation and vegetative biomass production as well as better nitrogen fixing ability. Therefore, initial investments in roots as a response to drought were found to improve performance of the plant under drought stress by paying off in more dry matter accumulation. Further, a direct relation between symbiotic nitrogen fixation and stomatal conductance, CO_2 assimilation and leaf dry mass was found. These suggest that, the relative growth of shoot vs. root were depends on the provision of nitrogen by symbiotic nitrogen fixation process by nodules and carbon by photosynthesis. Overall, lines BAT 477 and BT_34-1-1 were identified to be drought-tolerant, line RIL BT 6-1-1 to be only moderately tolerant and BT 51-1-1 was a drought escaper.

3.2   Introduction

Drought generally causes a decline in CO_2 assimilation, affects photochemical and biochemical reactions and restricts plant growth and dry matter accumulation (Chaves et al., 2002). Stomatal opening controls the gas exchange in plants and this is among the principal processes for plant adaptation to drought (Lawlor and Cornic, 2002). In common bean control of the stomatal opening is an adaptation strategy to overcome water deficit (Miyashita et al., 2005). Research has shown that restriction of leaf expansion, growth of young leaves and leaf senescence are further strategies in beans to adapt to drought conditions (White and Singh, 1991a). However, drought exposure ultimately results in a decrease of plant biomass and economic seed yield.

Common bean cultivars which confer better performance under drought were able to maintain higher tissue water retention capacity and attain higher biomass (Costa Franca et al., 2000). Gebeyehu (2006) reported a relative low reduction of leaf biomass by a tolerant bean cultivar when compared to a susceptible cultivar. This led to 29% reduction of the harvest index for the susceptible cultivar whereas the harvest index for the tolerant cultivar was unaffected. Also, deep rooting ability under water-limited condition (White et al., 1990), heliotropic leaf movement for protection from photoinhibition (Pastenes et al., 2005), early flowering or phenological adjustment (Acosta-Gallegos and White, 1994) and enhanced water and nitrogen use efficiency (Foster et al., 1995) under drought condition have also been found to be relevant in common bean for an adaptive or drought avoidance strategy.

Various bean varieties have been previously tested for their response to drought and results have been recently reviewed by Beebe et al. (2010). However, most of the studies mentioned in the review focused on shoot traits without considering the contribution of symbiotic nitrogen fixation (SNF). Nitrogen required for plant growth derives in legumes from SNF (Dakora and Keya, 1997). Among grain legumes, although common bean has relatively low nitrogen fixation ability it however contribute N for agricultural system by fixing nitrogen from 57kg N/ha (Herridge and Danso, 1995; Wani et al., 1995) to 100kg/ha (Hardarson et al., 1993). Drought is an important environmental factor affecting SNF (Serraj et al., 1999; Zahran, 1999a). Differential effects of bean cultivars under drought on SNF and biomass production have been previously reported (Castellanos et al., 1996a). SNF is often measured using the acetylene reduction assay (ARA), which is an indirect method for SNF determination, where the enzyme reducing N_2 to ammonia (nitrogenase) is also able to reduce acetylene to ethylene (Hardy et al., 1973; Turner and Gibson, 1980). Drought has been found to decrease plant biomass in beans by up to 35% and SNF by up to 80%. In the bean cultivar EMGOPA-201, a drought tolerant cultivar, dry mass was unaffected by growth at 50% soil water field capacity. However, number and mass of nodules as well as SNF decreased in this cultivar (Ramos et al., 1999) indicating that these processes are more sensitive to drought stress than biomass production. Therefore, reliable tools and indicators for tolerance of N_2 fixation in legumes to drought stress are indispensable for exploitation of genetic diversity of legumes. This would be achieved by understanding the effect of drought stress on SNF in relation to parameters at the whole plant level.

In previous soybean experiment, it has been ascertained that efficient SNF ability was associated with better gas exchange traits performance and accumulation of plant biomass. Thus, quick and

efficient allocations of plant biomass were considered to be as a result of enhanced SNF ability of the plant. These attributes in soybean were directly related with the better performance under drought (Fenta et al., 2011). However, whether this characteristics is common in other legumes needs to be confirmed.

So far, there is still little information available about plant performance parameters to determine drought tolerance in common bean. In particular, the contribution of SNF has been neglected in most common bean drought tolerance studies. In this study it has been hypothesized that morpho-physiological performance traits including nitrogen fixing ability would help for varietal performance evaluation in common bean. Therefore, the objective of this study was to determine under water deficit conditions the performance of different bean inbred lines with varying degrees of drought tolerance. This might allow identifying easily measurable plant performance parameters that are associated with drought tolerance of relevance for common bean. Furthermore, this study also sought to compare results with common bean with those obtained from soybean characterization under drought to identify widely applicable performance traits in legumes.

3.3   **Materials and methods**

3.3.1   Plant material and growth conditions

Plants of six common beans (*Phaseolus vulgaris* L.) with various phenotypes (Table 3.1) that have been obtained from the International Center of Tropical Agriculture (CIAT) were grown in

controlled environment phtotron at at Forestry and Agricultural biotechnology Institute (FABI), University of Pretoria (-25^0 45′ 20.64″S, 28^0 14′ 8.16″E) during spring season of 2009. The climatic condition of growth condition was, a day/night temperature of 25^{0}C / 17^{0}C and 60% relative humidity, 13 h photoperiod at the average light intensity of photosynthetically active radiation of 600 µmol m^{-2} s^{-1}. The light intensity was measured from 10 am to 3 pm using PAR 2 Meter with SW 11L sensor (S.W & W.S. Burrage, United Kingdom. Furthermore, the supplemental light with a capacity of 300 µmole m^{-2} s^{-2} was supplied with metal-halide lamps from 4:00 -7:00 pm. The environmental condition in the growth phtotron was monitored regularly to ensure the adequate growth conditions maintained.

One seed per pot was planted in 8 cm diameter pot and the emerging seedling was transferred to a 15.5 cm round pot with a volume of 218.20 cm^3 after two weeks or at the first trifoliate leaf (V1) stage. Seeds were inoculated before sowing with a *Rhizobium leguminosarum* biovar *phaseoli* powder (0.5 g per pot corresponding to 2.5x10^8 cells, Stimuplant CC., Pretoria, South Africa). Plants were grown in vermiculite fine grade (Mandoval PLC, Potchefstroom, South Africa). During the experimental period pots were rearranged periodically.

Table 3.1 Common bean lines used in this experiment including their background history

Line	Pedigree	Traits	Reference
BAT 477	(G3834 x G4493) x (G4792 x G5694)	Deep rooting ability	Sponchiado BN et al. (1989)
		Good N-fixer Fixing more N under drought	Hardarson G. et al. (1993) Castellanos et al.(1996a); Peña-Cabriales J. and Castellanos (1993)
DOR 364	(BAT 1215 x (RAB 166 x DOR 125)	Drought sensitive P-sensitive parent	Beebe et al.(1995) CIAT (1996)
BT 21138_34-1-1-M-M-M (BT_34-1-1)	RIL[1] (DOR 364 x BAT 477)	P-efficient	Drevon (unpublished)
BT 21138_147-3-M-M-M (BT_147-3)	RIL (DOR 364 x BAT 477)	P-inefficient	Drevon (unpublished)
BT 21138_6-1-1-M-M-M (BT_6-1-1)	RIL (DOR 364 x BAT 477)	Drought-adapted	CIAT (2007)
BT 21138_51-1-1-M-M-M (BT_51-1-1)	RIL (DOR 364 x BAT 477)	Drought-sensitive	CIAT yield trial (unpublished)

[1] RIL: Recombinant inbred line developed by single seed descent

3.3.2 Plant growth

Before the commencement of drought stress, plants were watered daily with N-free distilled water for up to two weeks and treated with a Hoagland's N-free nutrient solution every other day. Drought stress was initiated when plants were at the third trifoliate life stage (V3 stage) by completely withholding watering. For well-watered control plants the maximum water holding capacity was maintained by daily watering with Hoagland's N-free nutrient or distilled water throughout the experimental period. The maximum water holding capacity of the growth medium in this experiment was determined by watering equal amount of water to the well-watered pots and then allowing the medium to absorb until all micro and macro pores are filled for three hours and removing the remaining excess water from the saucer on the bottom of the pots.

3.3.3 Gas exchange

A portable Photosynthesis System (LI-COR using LI-6400/LI-6400XT Version 6, LI-COR Bioscience, Lincoln, USA) was used to measure the net photosynthetic assimilation rate, stomatal conductance, transpiration rate, leaf temperature, internal CO_2 concentration and Ci/Ca (intercellular CO_2/ambient CO_2) from the central leaflets of a fully matured 3rd and 4th trifoliate leaf. This was carried out by clamping a leaf into a leaf cuvette. Light intensity and CO_2 concentration inside the cuvette were maintained at 1000 $\mu mol\ m^{-2}s^{-1}$ and 400 $\mu mol\ mol^{-1}$, respectively, and the air temperature was kept at $25^{o}C$. The spot measurement was made on a 6 cm^2 leaf area and measurements started at commencement of drought treatment until the

assimilation rate approached almost zero (18 days of drought treatment). These measurements were conducted by sampling four individual plants from each water treatment.

3.3.4 Leaf water potential

The central leaflet used for gas exchange measurement was also used for measuring the leaf water potential. Measurement was carried out by using a pressure bomb (Model 3005, ICT International, Armidale, Australia) according to the method of (Mario Valenzuela-Vazquez et al., 1997). Since measurement was destructive to the leaf, measurements were made only at three time points during the drought treatment.

3.3.5 Soil water content

To determine the soil moisture content, vermiculite samples were taken every other day from all potted test plants by using a cylindrical core borer (1.4 cm in diameter and 11 cm long). The fresh mass of vermiculite sample was measured immediately by using a balance with an accuracy of 0.001 g (Model B-502-S, METTLER TOLEDO, Greifensee, Switzerland). Samples were placed for drying into an oven (Type U 40, Mommert, Schwabach, Germany) at a temperature of $60^{o}C$ for 48 hrs. The water mass (water mass) was calculated (percentage) as the difference between the mass of the wet and oven-dried vermiculite samples.

3.3.6 Biomass and leaf area

For quantifying the effect of drought stress on biomass accumulation four individual plants (replicates) from each bean lines were harvested and above-ground parts of the plant were separated into leaves (with petioles), stems and pods. Below-ground parts (root and nodules) were separately harvested and the fresh mass determined. Before oven drying, the leaf area per plant was measured by using leaf area meter LI-COR 3100 (LI-COR Inc., USA). Dry mass was obtained from oven dried samples after drying plant material at $60^{o}C$ for 48 hrs. After drying, dry mass of each sample (leaf biomass, stem biomass, pod biomass, and root biomass) was measured to determine total dry matter production.

3.3.7 Symbiotic nitrogen fixation (SNF)

SNF potential was estimated using the acetylene reduction assay (ARA) method which is an indirect method for SNF determination as described by Hardy et al. (1973) and Turner and Gibson (1980). All crown and lateral nodules of four individual plants for each cultivar were harvested and after the mass as well as nodule number recorded, the nodules were assayed for acetylene reduction. Nodules were placed in an airtight small flask of 43 ml capacity and ethylene production was determined after 10 minutes incubation with 4 ml acetylene and injecting 1 ml of gas from each flask into a gas chromatograph Varian 3900 (Varian inc., USA). The oven temperature was maintained at 80°C, FID detector: 200, 1177:180^{0}C, Gas flow: air (300), H_2 (30), N_2 carrier gas (25) and running time was 4.8 minute. For calibration, a standard curve was made by injecting ethylene.

3.3.8   Statistical analysis

All data were analyzed using the JMP® 9 (2011, SAS Institute Inc., Cary, NC, USA) statistical package. Analysis of variance was carried out for determining significant differences in performance between the tested bean lines. Least Squares Means (LSmeans) Student's t-test (P= 0.05) was used for treatment comparison. Multivariate Pearson's correlation analysis was used for determining the relationship (correlation) between measured traits. The pooled data of all lines and for the entire measurement period were used for analysis of correlation. Principal component analysis (PCA) on correlation was also performed.

3.4   **Results**

3.4.1   Vermiculite water content and leaf water potential

The vermiculite (soil) water content was determined on a mass basis and the value shown in Figure 3.1, is the percentage difference of the mass of wet and oven dry vermiculite. During the initial period of drought stress (first week of drought), the moisture content of vermiculite was not different for all tested lines. However, 15 days after drought, vermiculite water content for plants of lines BT_34-1-1 and BAT 477 (39%) was significantly lower than plants of the remaining tested lines (Figure 3. 1).

Due to the decline of moisture content in vermiculite, the leaf water potential also declined in all plants. Plants of BT_147-3 and DOR 364 had a significantly lower (P<0.05) leaf water potential after 10 days of drought than plants of all other lines (Figure 3.2). Plants of BAT 477 and BT_34-1-1 exhibited the highest water potential, although not significantly different (P > 0.05), and also their soil water content was the lowest when compared to all other lines at the end of the experimental period. This suggests that plants of these two lines maintain their water status due to higher absorption of water by their roots.
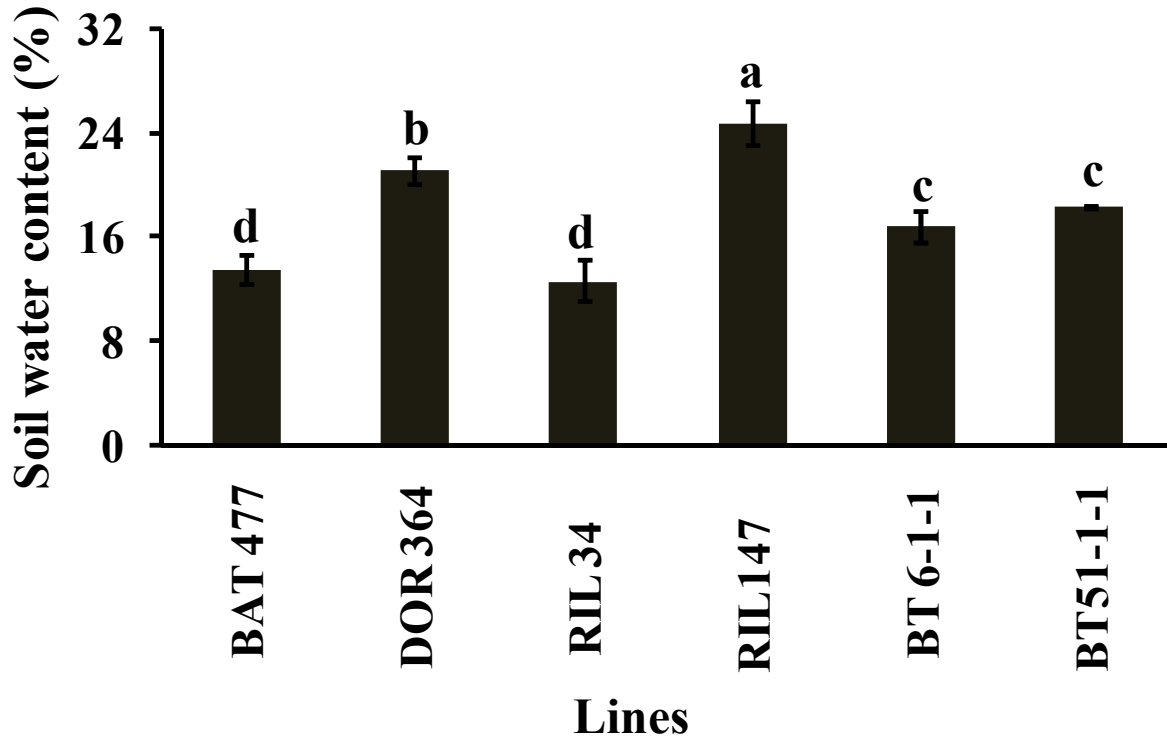
Figure 3.1 Effect of six common bean lines on vermiculite water content after the plants were exposed to water stress for 15 days. Each bar represents the mean ± SE from four individual plants. Different letter on bar denote significant difference (P < 0.05). The value indicates the calculated result of the percentage difference of the mass of wet and oven dry vermiculite sample.
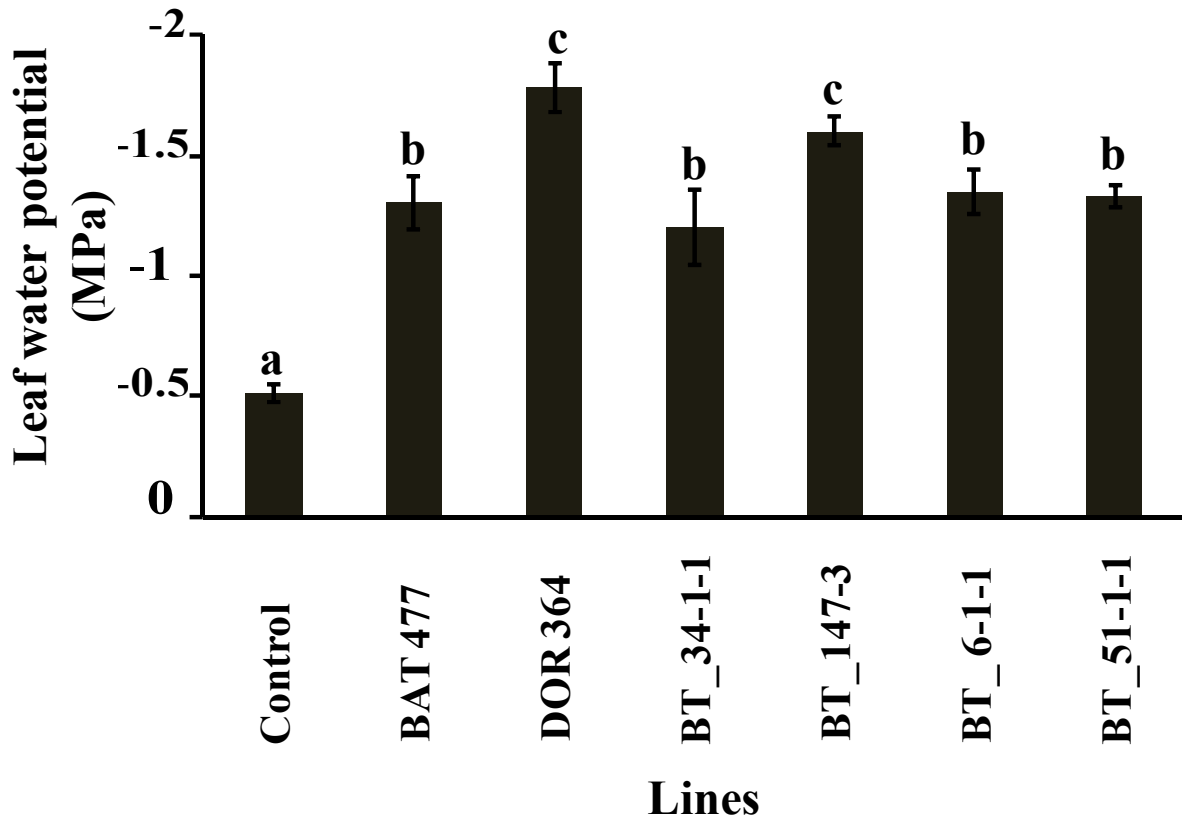
Figure 3.2 Effect of water deprivation on leaf water potential value (MPa) for plants of six bean lines grown under drought. Values represent the mean ± SEM of four individual plants grown under drought for 10 days. Control represents the mean ± SEM of 24 pooled plants (4 plants for each line) grown under well-watered conditions. Different letter on bar denote significant difference (P < 0.05).

3.4.2   Effect of drought on gas exchange

For gas exchange traits significant differences were not found for analysis of variances for two ways ANOVA (data not shown), however significance differences were revealed for one way ANOVA (Appendix 2). As a result main effects of these performance parameters will be discussed in this result. Accordingly, analysis of variance conducted for measurements carried out over the whole experimental period showed that plants of tested lines significantly differed in CO_2 assimilation and stomatal conductance under drought and also well watered conditions. At the onset of drought stress, both the stomatal conductance and CO_2 assimilation were not significantly different ($P > 0.05$) for plants of all tested lines (Tables 3.2 and 3.3). However, after 7 days of drought, the highest stomatal conductance was measured in plants of BT_34-1-1 and BAT 477 (Table 3.2). These lines had their stomata open and they also had the highest photosynthetic gas assimilation (Table 3.3). In contrast, the lowest stomatal conductance after 7 days of drought was measured in plants of lines BT 51-1-1 and BT_147-3 that closed their stomata under drought and they also had the lowest CO_2 assimilation (Table 3.3). A similar trend of highest and lowest stomatal conductance and CO_2 assimilation in the plants of the different lines tested was also found after 18 days of drought (Tables 3.2 and 3.3). In addition, plants of line BT 6-1-1 had also a similar high stomatal conductance and CO_2 assimilation after 18 days of drought comparable to BAT 477 and BT_34-1-1.

Under well-watered conditions, plants of all tested lines had similar IWUE values (data not shown). The average value of IWUE for all bean lines (40 μCO_2/mol H_2O) at well-watered condition (Figure 3.3), which was 33% and 125% less from the susceptible and tolerant cultivars

respectively. After 15 days of drought, plants of DOR 364 and BT_147-3 had the lowest IWUE when compared to the other lines (Figure 3.3). However, IWUE was not significantly different (P > 0.05) to the other lines except for BT_34-1-1 which had a significantly higher (P < 0.05) IWUE than DOR 364. In general, line BT_34-1-1 had the highest IWUE of all lines tested and plants of this line are therefore are able to assimilate more CO_2 per unit of stomatal conductance than plants of other lines under water deficit conditions.

According to the association of gas exchange parameters (A, G. and CI) each other for the tested bean lines, data for individual cultivars and for the pooled data, highly significant positive association (P<0.01) was observed between A and G both under well-watered as well as under drought stress conditions. However, although the correlation of G with Ci was positive under both well-watered and drought conditions, the correlation analysis of Ci with photosynthesis was positive and highly significant (P<0.01) under drought condition nevertheless, this relationship was not significant under well-watered condition (data not shown).

Table 3.2 Comparison of stomatal conductance in six common bean lines at different time intervals under drought conditions. Data are the means ±SEM of four different plants per line for each date. Different letter within a column denote significant difference (P < 0.05).

Lines	Stomatal conductance (mmol $m^{-2}s^{-1}$)			
	0 day	**7 days**	**15 days**	**18 days**
BAT 477	662.7±47.4	492.7±16.3ab	49.1±7.1a	24.2±1.4ab
DOR 364	578.3±81.2	184.8±15.4c	18.8±0.8c	3.0±0.3c
BT_34-1-1	765.0±17.9	576.9±25.3a	31.8±1.2ab	28.8±2.0a
BT_147-3	572.7±34.3	167.6±13.1c	21.2±1.8c	3.6±0.5c
BT 6-1-1	651.3±85.5	399.4±24.2b	34.9±3.4ab	16.7±3.8b
BT 51-1-1	585.0±15.5	164.8±14.2c	24.9±9.0ab	2.5±0.3c
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

Table 3.3 Comparison of photosynthetic assimilation in six common bean lines at different time intervals under drought conditions. Data are the means ±SEM of four different plants per line. Different letters within a column denote significant differences ($P < 0.05$).

Lines	CO_2 assimilation ($\mu mol\ m^{-2}s^{-1}$)			
	0 day	7 days	15 days	18 days
BAT 477	14.16±0.17	7.91±0.22a	4.03±0.53a	0.88±0.14a
DOR 364	13.33±0.33	5.64±0.39bc	1.11±0.3b	0.11±0.05b
BT_34-1-1	15.15±0.09	7.59±0.24a	3.15±0.4a	0.87±0.05a
BT_147-3	13.43±0.42	4.23±0.25cd	1.27±0.19b	0.16±0.09b
BT 6-1-1	14.42±0.53	6.78±0.3ab	2.98±0.13ab	0.55±0.05ab
BT 51-1-1	14.05±0.23	3.97±0.28d	2.22±0.17b	0.10±0.11b
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
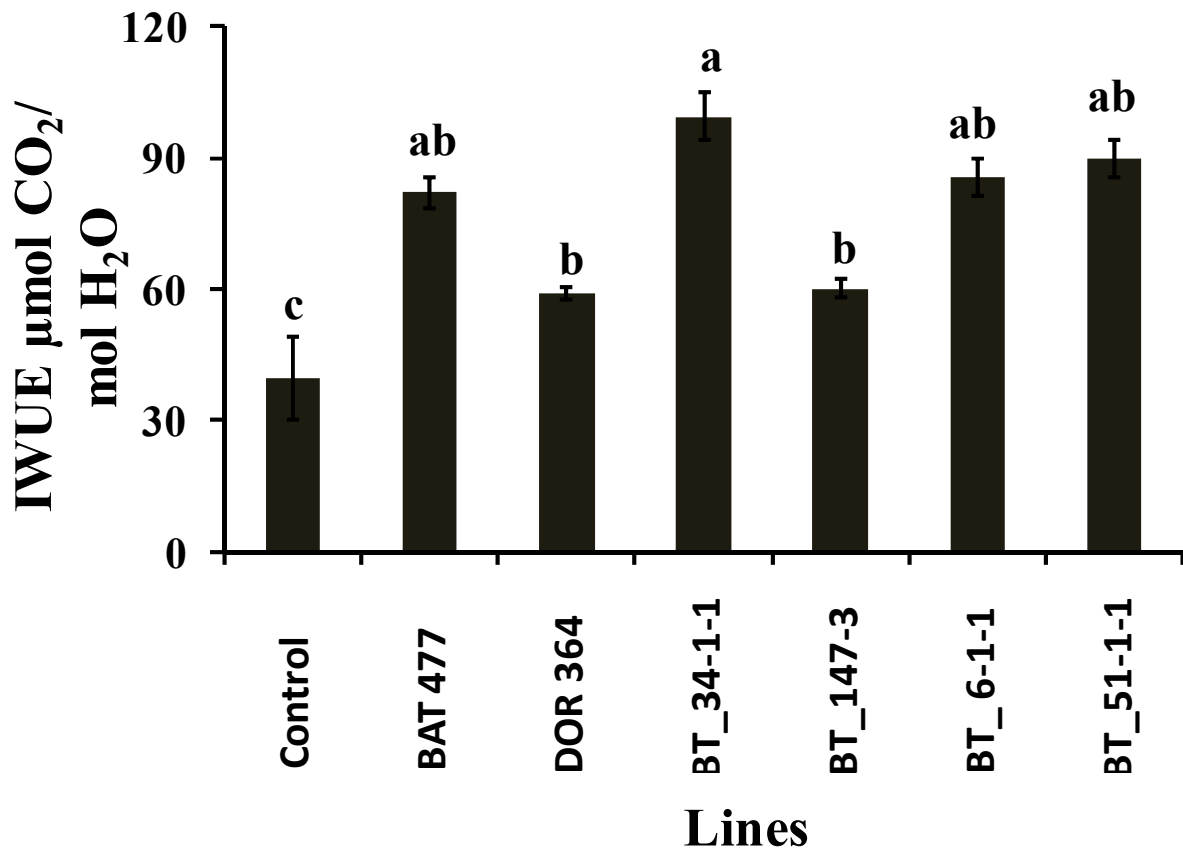
Figure 3.3 Comparison of instantaneous water use efficiency (IWUE) values measured in six common bean lines after 15 days of drought treatment. Data are the means ±SEM of four different plants per lines grown under drought condition. Control represents the mean ±SEM of 24 pooled plants (4 plants for each lines) grown under well-watered conditions. Different letter on the bar denote significant difference (P<0.05).

3.4.3 Drought effect on plant development and biomass distribution

For measurements taken over the entire experimental period, the analysis of variance showed that plants of tested lines significantly differed for leaf, stem and root dry mass under both drought and well-watered conditions (Appendix 2), however, two way ANOVA (water treatment X lines) for biomass traits were not significant (data not shown). Drought treatment reduced the total biomass (leaf, stem, pod and root) of plants, but there was no significant difference (P > 0.05) between plants of different lines after identical treatment (well-watered or drought) (Table 3.4). Drought stress reduced the shoot biomass (leaf and stem) when compared to well-watered control plants. The most significant reduction in shoot biomass was found after 18 days of drought in plants of lines DOR 364 and BT_147-3 (about 80% reduction). Plants of all other lines had only a 60-69% reduction in shoot biomass. However, there was no significant difference (P > 0.05) between pod biomass of plants of different lines after identical treatment (well-watered or drought) (Table 3.4).

In contrast, drought stress increased the root biomass in all plants of the different lines tested (Table 3.4). The highest root biomass was found in BT_34-1-1 and the lowest in BT_147-3 and DOR 364. However, root biomass of BT_34-1-1 was only significantly different (P < 0.05) to the root biomass of DOR 364 and BT_147-3. And similar response of lines was measured for the root/shoot ratio with the highest in line BT_34-1-1 and the lowest in lines BT_147-3 (Table 3.4)

In well-watered conditions the leaf area of tested lines did not differ significantly (P > 0.05) (Table 3.4). After 15 days of drought, plants of the three lines BAT 477, BT_34-1-1 and BT 6-1-1

had the highest leaf area. The lowest leaf area was measured in DOR 364 which was significantly

lower ($P < 0.05$) than the leaf area in lines BAT 477, BT_34-1-1 and BT 6-1-1 (Table 3.4).

Table 3.4 Dry mass (g) of plant parts, root / shoot (leaf and stem) dry mass ratio, and leaf area (m^2), of plants of six common bean lines after 18 days and leaf area after 15 days of exposure to drought or well-watered conditions. Data represent the mean ± SEM of four independent plants per line. Different letter within a column denote significant difference (P < 0.05).

A) <u>Well-watered</u>

Lines	Dry mass					Root/shoot	Leaf area
	Leaf	**Stem**	**Pod**	**Root**	**Total**		
BAT 477	3.24±0.53a	1.72±0.33ab	1.61±0.47	1.67±0.22a	6.57±1.39	0.25±0.05	13.47±0.74
DOR 364	2.03±0.42b	1.29±0.3ab	1.41±0.55	1.05±0.17b	4.99±1.25	0.21±0.05	10.68±0.95
BT_34-1-1	3.33±0.54a	2.16±0.55a	1.85±0.53	1.77±0.24a	7.34±1.51	0.24±0.06	12.12±0.88
BT_147-3	1.96±0.25b	1.32±0.31ab	2.44±0.67	1.12±0.14b	5.71±1.26	0.20±0.05	10.78±0.81
BT 6-1-1	2.84±0.67ab	1.81±0.54ab	2.17±0.73	1.54±0.18a	6.82±2.08	0.23±0.06	12.99±1.15
BT 51-1-1	2.33±0.42ab	1.12±0.34b	2.32±0.96	0.98±0.17b	5.47±1.78	0.18±0.04	12.75±1.16
Significance	*	**	Ns	**	ns	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

B) <u>Drought</u>

Lines	Dry mass					Root/shoot	Leaf area
	Leaf	Stem	Pod	Root	Total		
BAT 477	1.98±0.33a	1.34±0.27ab	1.48±0.36	2.99±45ab	4.80±1.2	0.62±0.03ab	9.90±0.21a
DOR 364	1.62±0.35b	1.06±0.23b	1.08±0.29	2.22±0.61b	3.77±1.35	0.59±0.03b	7.48±0.43c
BT_34-1-1	2.28±0.26a	1.66±0.24a	1.38±0.44	3.74±0.65a	5.32±1.25	0.70±0.02a	9.60±0.47ab
BT_147-3	1.58±0.13b	0.97±0.11b	1.29±0.44	2.22±0.46b	3.85±0.96	0.57±0.03b	8.05±0.24bc
BT 6-1-1	1.75±0.24ab	1.12±0.22b	1.59±0.26	2.64±0.48ab	4.46±1.13	0.59±0.03ab	9.36±0.21ab
BT 51-1-1	1.61±0.25b	0.96±0.18b	1.55±0.43	2.62±0.66ab	4.13±1.41	0.63±0.02ab	8.19±0.34bc
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

3.4.4   Nodule performance and symbiotic nitrogen fixation (SNF)

Since the colour of nodules changed to green after 18 days of drought (indicating that nodules were inactive), SNF measurements were carried out only for 7 and 10 days after drought exposure and data from the two points were pooled. According to the analysis of variance, bean lines had significant differences for both nodule fresh mass and SNF under drought and SNF under well-watered conditions (Appendix 2), nevertheless, significant interactions of lines vs. water treatment were not shown for these nodule performance traits (data not shown). Under well-watered conditions plants of BAT 477 and BT_34-1-1 had the highest and plants of DOR 364 had the lowest nodule fresh mass which was significantly ($P < 0.05$) different (Figure 3.4). Under drought, BAT 477, BT_34-1-1 and BT 51-1-1 exhibited the highest nodule fresh mass, and line BT 6-1-1 the lowest, being was significantly ($P < 0.05$) different to lines BAT 477 and BT_34-1-1 (Figure 3.4).

Marked differences were also found among the lines for SNF under well-watered and drought conditions. Comparable to the result found for nodule fresh mass, lines BAT 477 and BT_34-1-1 had the highest SNF BT_34-1-1 under both well-watered and drought conditions with the highest SNF found under drought in line BT_34-1-1 (Figure 3.5). In this line SNF was significantly ($P < 0.05$) higher under drought to SNF measured in lines DOR 364 and BT_147-3 (Figure 3.5).
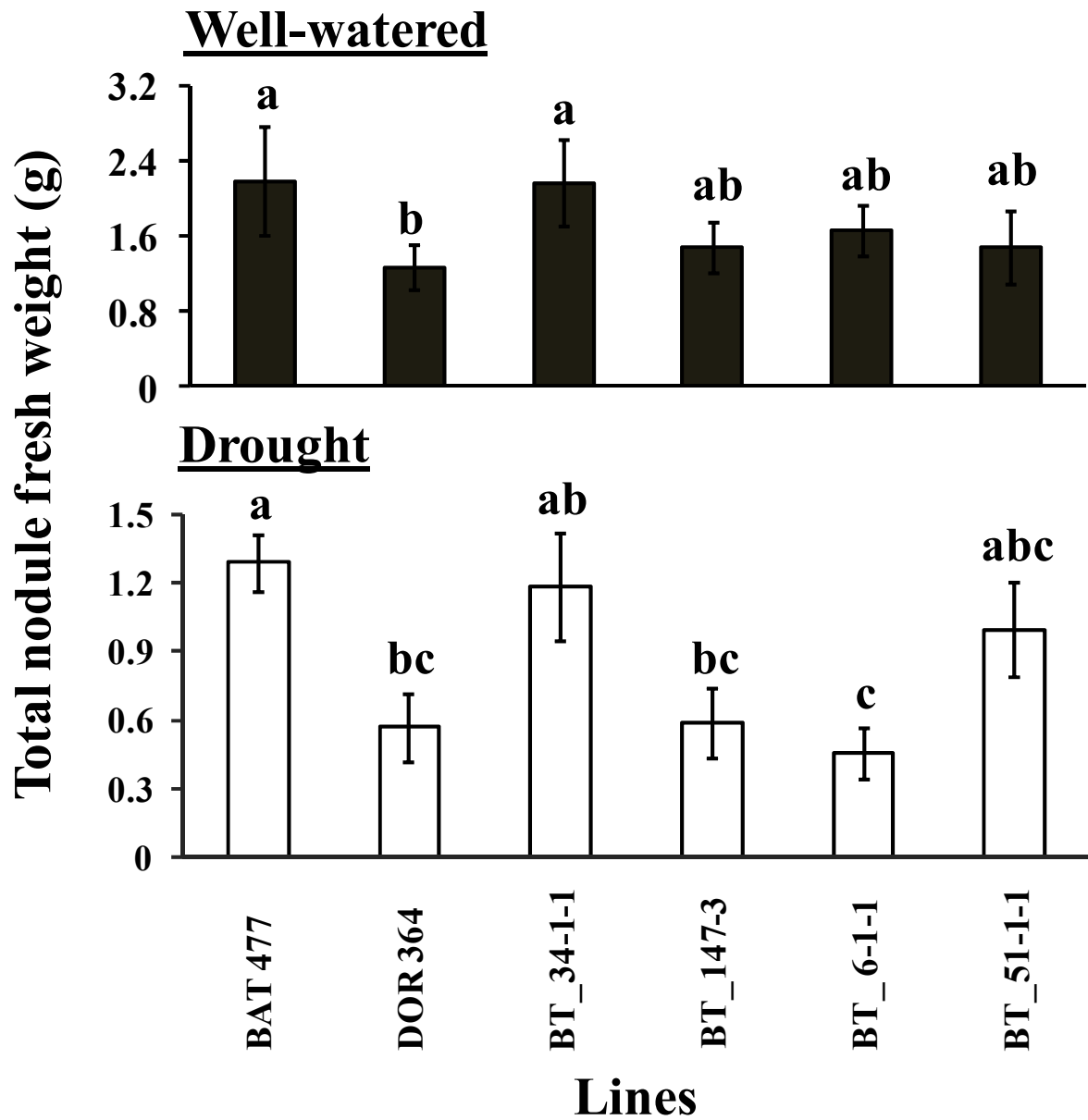
Figure 3.4 Nodules fresh mass of plants of six different bean lines grown either under well-watered or drought conditions. Data represent the mean ± SEM of 4 individual plants. Measurements were carried out 7 and 10 days after exposure of plants to drought and well-watered conditions and individual data obtained from the two time points were pooled. Different letter on bar denote significant difference ($P < 0.05$).
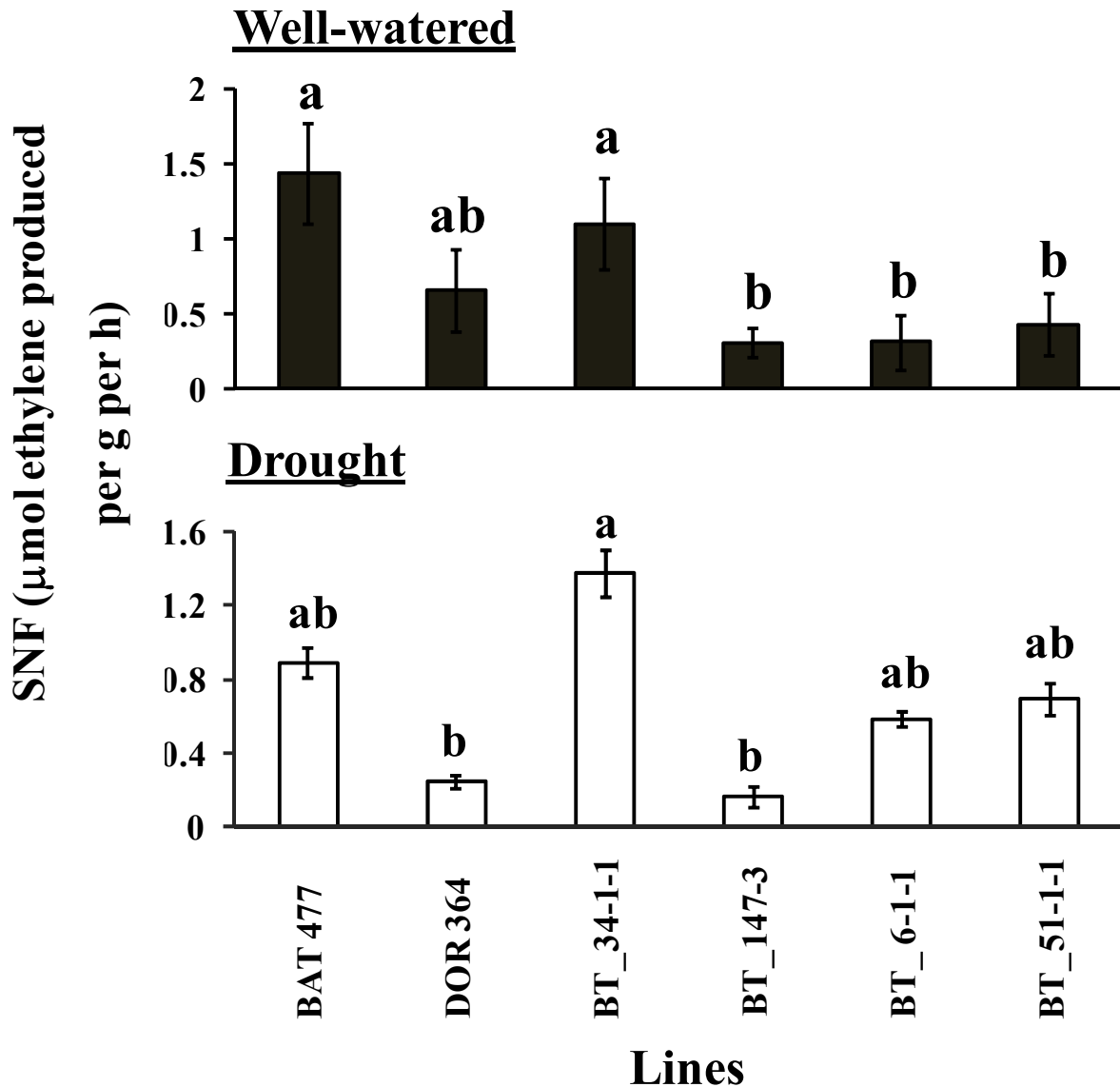
Figure 3.5 Nodule SNF of plants of six different bean lines grown either under well-watered or drought conditions. Bars represent the mean ± SEM of 4 individual plants. Measurements were carried 7 and 10 days after exposure of plants to drought and well-watered conditions and individual data obtained from the two time points were pooled. Different letter within a column denote significant difference (P < 0.05).

3.4.5   <u>Nodule performance association with growth and gas exchange</u>

Under well-watered conditions there was a positive and significant (P < 0.05) association between nodule fresh mass and leaf and root dry mass as well as for gas exchange parameters (CO_2 assimilation, stomatal conductance, intra-cellular CO_2 concentration) (Table 3.5). Under drought, a positive significant (P < 0.05) association was found between nodule fresh mass and gas exchange parameters identical to the well-watered conditions (Table 3.5). In contrast, a significant (P < 0.05) negative association was between nodule fresh mass with total shoot and root dry mass in drought growth conditions (Table 3.5).

When an association between SNF and various traits was determined under well-watered conditions, a positive (P < 0.05) association was found between SNF and root dry mass as well as gas exchange parameters (CO_2 assimilation, stomatal conductance, intra-cellular CO_2 concentration) (Table 3.5). Under drought, an identical positive significant (P < 0.05) association existed between SNF and gas exchange parameters. There was also a positive significant (P < 0.05) association between SNF and leaf area.

To explore the sources of variation in different bean lines, data of ten performance traits measured over the whole experimental period were used for principal component analysis (PCA). PCA is a technique for reducing the complexity of high-dimensional data, to approximate that data with fewer dimensions. In PCA the variance of data is captured in a low-dimensional sub-space (quadrant, Figure 3.6) to understand the sources of variation in data. Each dimension is called a principal component (arrows in the quadrant, Figure 3.6). This component represents a

linear combination of the original variables (JMP®8.02, 2011). It helps to clearly visualize the arrangement of the parameters used in the study. A biplot (all results in Figure 3.6) in the PCA helps to display both the observations and variables of multivariate data in the same plot. The variables are shown as arrows in the plot. These arrows called biplot rays, approximate the variables as a function of the principal components on the axes and the rays represent the variables. The length of the ray corresponds to the eigenvalue or variance of the principal component with shorter arrows being less significant and longer arrows highly significant. The eigenvalues represent a partition of the total variation in the multivariate sample (JMP®8.02, 2011). Further, the "Factor" mentioned in Figure 3.6 represents the percentage of variation of the arrows of the analyzed parameters.

In Figure 3.6 the two principal components (Factor 1 and 2) account for approximately 65% of the total variability between the tested lines under drought conditions and 54.9% under well-watered condition. This means that under drought there is higher variability of measured traits than under well-watered conditions. According to the PCA analysis (Figure 3.6A and Table 3.6), under well-watered condition (Factor 1 = % of variation) leaf, root and total shoot dry mass, as well as leaf area contribute by 29.8% to the total variation. For Factor 2, gas exchange parameters (A, G, and CI) and SNF/g of nodules contributed with 25.1% to the total variation. For both Factors the values of eigenvector were positive indicating a positive contribution of these traits to overall performance (Figure 3.6A and Table 3.6). Under drought, for Factor 1 A, G, nodule fresh mass and SNF/g of nodules contributed with 38.5% to the total variation. For Factor 2 dry mass of leaf, root and total shoot as well as leaf area were contributed with 26.5% to the overall variation. Except leaf temperature, all parameters had a positive eigenvector

contributing positively to performance under drought (Figure 3.6B and Table 3.6). Further, under both conditions, well-watered and drought, SNF highly correlates with A and G in the same quadrant indicating that A and G contributed for SNF and also *vice versa* (Figure 3.6).

Table 3.5 Association of growth and gas exchange parameters with nodule fresh mass (FW) or SNF using Pearson's ρ correlation analysis under drought and well-watered conditions using pooled data (days 0, 7, 10, 15 and 18) from plants of all lines.

Trait	Trait	Well-watered		Drought	
		r	P-value	r	P-value
	Leaf area	0.084	0.6749	-0.150	0.4515
	Leaf DW	0.366	0.0240*	-0.112	0.8736
	Root DW	0.502	0.0003**	-0.567	0.0010**
Nodule FW	Total shoot DW	0.158	0.7502	-0.624	0.0214*
	CO_2 assimilation	0.463	0.0041**	0.873	0.0001**
	Stomatal conductance	0.325	0.0018**	0.885	<.0001**
	CI	0.244	0.0001**	0.338	<.0001**
	Leaf temperature	0.075	0.4335	-0.507	0.0670
	Leaf area	0.046	0.2992	0.045	0.0378*
	Leaf DW	0.266	0.0942	0.159	0.0874
	Root DW	0.379	0.0006**	-0.273	0.7182
SNF	Total shoot DW	0.016	0.1522	0.059	0.7316
	CO_2 assimilation	0.472	<.0001**	0.544	<.0001**
	Stomatal conductance	0.545	<.0001**	0.638	<.0001**
	CI	0.36	0.0075**	0.307	0.0161*
	Leaf temperature	0.093	0.2322	-0.231	0.5542

NOTE: r = Pearson's ρ correlation coefficient

CI= Intracellular CO_2 concentration

DW= dry mass

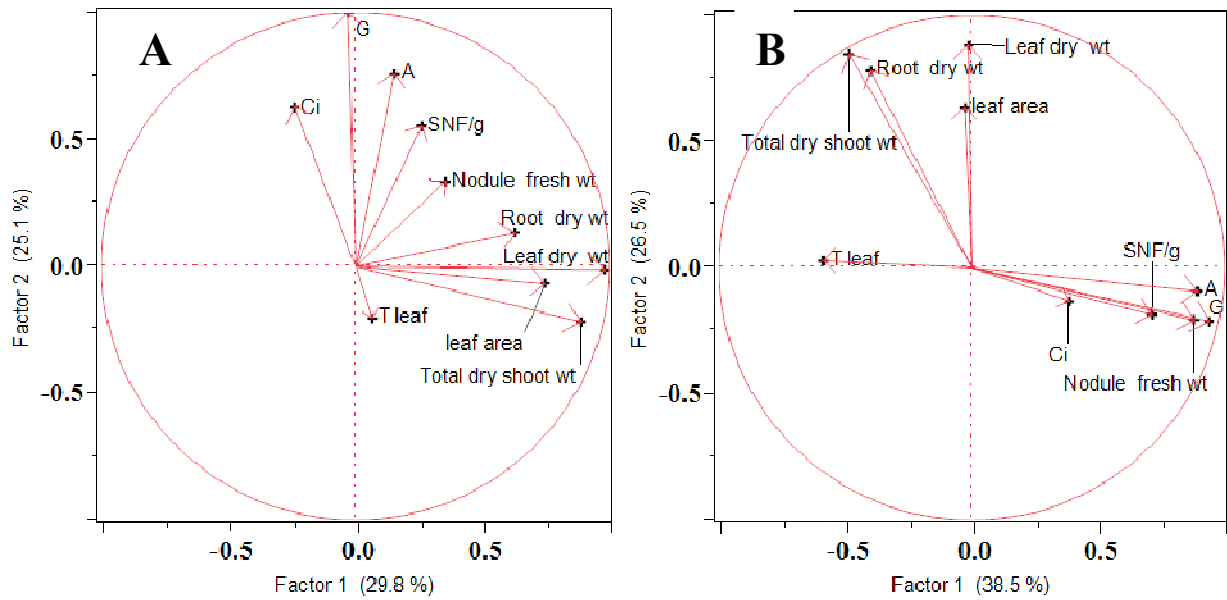SNF=symbiotic nitrogen fixation (ARA/g of fresh nodule mass)

Figure 3.6 Principal component analysis and factor loading plot data of pooled data of the entire measurement period for 10 performance parameters of bean under well-watered (A) and drought (B) conditions.

Table 3.6 Factor analyses of 10 performance traits where "Factors" represent the percentage of variation in the biplot and numbers in table indicate the distance of the vectors shown in the biplot (Figure 3.6).

Traits	Drought		Well-watered	
	Factor 1	Factor 2	Factor 1	Factor 2
Leaf DW	-0.015	0.883	0.980	-0.013
Root DW	-0.401	0.779	0.627	0.132
Leaf area	-0.029	0.635	0.746	-0.069
Total shoot DW	-0.485	0.846	0.887	-0.219
A	0.893	-0.093	0.154	0.761
G	0.939	-0.213	-0.029	0.999
Ci	0.384	-0.132	-0.240	0.629
T leaf	-0.587	0.027	0.066	-0.204
Nodule FW	0.882	-0.209	0.354	0.336
SNF	0.711	-0.186	0.260	0.553

3.5   **Discussion**

This part of the study has shown that all measured performance traits in plants of different bean lines were affected by drought stress with gas exchange parameters (stomatal conductance and CO_2 assimilation) and SNF as the most sensitive. This confirms the previous findings with soybean reported in chapter two and previous results where SNF in soybean (Sinclair, 1986) and common bean (Castellanos et al., 1996a) cultivars were greatly decreased relative to the leaf gas exchange activity due to the effect of drought stress.

The positive and strong association of stomatal conductance with CO_2 assimilation under both water regimes suggests the limitation of CO_2 assimilation during drought stress is mainly governed by stomatal conductance. The positive association of Ci and photosynthesis suggests the decline in CO_2 assimilation under water stressed condition is mainly associated with limited CO_2 fixation due to stomatal limitation as it has been also suggested before by Chaves and Oliveira (2004). However, without measurements at elevated CO_2 concentrations (Lawlor, 2002a; Lawlor and Tezara, 2009b; Tezara et al., 1999) the relative effects of stomatal and mesophyll effects cannot be determined. As Ort et al. (1994) outlined, decrease in Ci plays a leading role in mediating in the change in biochemical activity during drought. Ci decrease will result in reduction of CO_2 assimilation by Rubisco and enhancing photorespiration (Medrano et al., 2002). Evidence is (Lawlor and Tezara, 2009b; Tezara et al., 1999) that a decrease in ATP synthase is an early effect of cellular water deficit. This leads to decreased ATP and decreased RuBP synthesis, slower CO_2 fixation (i.e. photosynthesis and photorespiration). Decreased CO_2 assimilation means that the energy captured ion the thylakoids is not used. This results in

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decreased pH in the thylakoids and greater trans-membrane potential (Osmond et al., 1997; Pfündel and W. Bilger, 1994) and xanthophyll de-epoxidation will follow. These will result in an increase in the photochemical quenching and heat dissipation at the antenna, steady state chlorophyll florescence will drop (Medrano et al., 2002; Pfündel and W. Bilger, 1994). The enzyme sucrose phosphate synthase which has a key function in source-sink relations (Chaves and Oliveira, 2004; Vassey and Sharkey, 1989) may be inhibited by water deficit, and this may reduces the starch content. Also, the changes in ATP may also alter gene expression of the plant (Chaves and Oliveira, 2004).These, findings reveals the importance of maintaining CO_2 assimilation under water stress. However, the similar positive and significant association of Ci and photosynthesis for both better performer and susceptible bean lines under drought suggests a decline in Ci under drought stress may not only due to decrease in stomatal conductance. In such cases non-stomatal (metabolic) limitations to photosynthesis could be a factor which should be taken in to consideration (Tezara et al., 2002; Tezara et al., 2003). However, there is a uncertainty in the calculations and the use of Ci vs. CO_2 assimilation association as an indicator for stress evaluation due to patchy (irregular) stomatal closure (Buckley et al., 1997) and the existence of cuticular transpiration at the time of stomata closed (Boyer et al., 1997) under drought condition. This suggests the importance of complimenting gas exchange data with other physiological traits.

The ratio between assimilation and stomatal conductance, IWUE is also a good parameter for selecting superior performing legume cultivars (Fenta et al., 2011). Based on IWUE analysis three cultivars (BAT 477, BT_34-1-1, and BT 6-1-1) showed superior performance also under drought. Attaining double merit in gas exchange efficiencies (CO_2 assimilation and IWUE) might benefit the two lines (BAT 477 and BT_34-1-1) for better performance under drought

condition than other lines. Drought also impairs the carbon assimilation through biochemical and photochemical effects (Chaves et al., 2002). According to Gimenez et al. (1992), there is a strong correlation between CO_2 assimilation and RUBP. Water deficit affects the photosynthetic enzymatic activity, especially RUBP. The rate of RUBP synthesis is the prominent factor affecting CO_2 assimilation (Lawlor and Cornic, 2002) depending on the synthesis of NDPH and ATP. ATP deficiency and changes in proteins in the leaf are key factors for the loss of Rubisco activity (Lawlor and Tezara, 2009b). Therefore, those bean lines with a better CO_2 assimilation and IWUE might also able to supply ATP maintaining the cellular enzymatic activity and important leaf proteins.

Maintaining the leaf water status of the plant, as a trait, was the major characteristic in common bean to provide drought tolerance. The leaf water status was directly related to stomata opening and production of shoot and root biomass as well as SNF. The best performing lines in this study were BAT 477 and BT_34-1-1. Both had higher stomatal conductance and photosynthetic CO_2 assimilation when compared to the other four lines. These two best-performing lines responded rapidly to drought stress with an enhanced root development resulting in a better shoot biomass. Enhanced root development will provide better water-uptake, such that plants will keep stomata open allowing better CO_2 assimilation. This will result in higher biomass production. Such a response in bean has also been reported by Yadav et al. (1997). Further, it is well-documented that stomatal opening and closing, which depends on the leaf water status of the plant, are regulated by growth hormones such as abscisic acid (ABA) (Kim et al., 2010). This hormone has also been found to enhance lateral root development in the legume *Medicago* (Liang and Harris, 2005).

Enhanced root mass has the advantage for production of higher shoot biomass and ultimately higher seed yield (Sinclair and Muchow, 2001). The transport of reserves in the plant (sink strength) depends up on the accessibility and translocation of water in the plant parts. Maintaining a higher root to shoot ratio is also a prominent performance attribute for better under drought. In this study, four lines (BAT 477, BT_34-1-1, BT_6-1-1 and BT_51-1-1) exhibited higher root to shoot ratio and performed better under drought (Table 3.5). However, while typical reduction of shoot development due to drought is common, there is a increase in dry matter distribution in the root portion improving the root/shoot-ratio (Wilson, 1988). Increase in root/shoot biomass ratio under water limited condition has been observed in different crops such as soybean (Fenta et al., 2011; McCoy et al., 1990), spring wheat (Li et al., 1994) and Brassica juncea (Rabha and Uprety, 1998).These observations reveled that maintaining functional balance between root and shoot is a crucial attribute for better performance under drought stress condition.

According to Hay and Porter (2006), variation of water absorbed by the plant over the growing time further depends on the capability of the roots to extract the water per unit volume of soil/growth media. The leaf water potential will be lowered due to transpiration creating a gradient in water potential. This helps to move the water from the soil to the root. Extended roots in to the growth media and transport of water to the canopy would be achieved only when the water potential of the root xylem is lowered by transpiration or stomatal opening. Lines with better root biomass, root/shoot ratio and higher biomass possibly have such characteristics which results in enhanced performance under drought. Therefore, initial investment in roots as a

response to drought will improve performance of the plant under drought stress and hence will pay off in more shoot and productivity as also suggested by DaCosta and Huang (2006).

Drought reduced shoot biomass in plants of all bean lines and the degree of reduction was comparable to the reduction in leaf area. According to the observation made on the water stressed and well-watered plants, the decline in leaf area was due to the fewer leaves as well as smaller leaves. This is due to the fact that drought inhibits the expansion of the existing leaves and the regeneration of the new emerging leaves however, these effects were severe in the susceptible cultivars.In contrast, the root mass increased in all lines under drought.

According to Blum (2011), effective use of water (EUW) is defined as "enhancement of biomass production under drought stress primarily by maximizing soil water capture while diverting the largest part of the available soil moisture towards stomatal transpiration." This EUW is a stress adaptive trait which helps for osmotic adjustment and sustains the stomatal conductance and eventually for enhanced CO_2 assimilation. It has been suggested that deep root system was allowed for better water absorption and water use through deep and dense root was also associated with higher productivity and drought tolerance (Pinheiro et al., 2005). Therefore, a variety which shows with a better performance for maximizing the water absorption through root development and convert the absorbed water to plant productivity and avoid water stress can be termed as it has better EUW.

The ability of a particular plant to transport the photo-assimilates to the plant organ for dry matter production (biomass or harvestable yield) of the plant is termed as portioning ability. The transport of assimilate depend on the sink strength and the growth condition of the plant which

varies according to the performance of a specific cultivar (Zhang et al., 2005). However, since assimilate is a limited source during water stress condition, the pattern of supply of assimilate to which to specific part of plant organ (sink) or the pattern of assimilate distribution has always a debate. Nevertheless, according to functional balance analysis, carbon assimilation by the shoot and root occurs according to the highest rate of return (i.e., the relative increase of dry matter accumulation in response to partitioning of one unit of assimilates) (Brouwer, 1962). Thus, effective balancing of assimilate to the root and to shoot under water-limited condition would be advantageous by maintaining shoot: root ratio for sustaining respiration.

Although gas exchange parameters, leaf water potential and biomass production decreased in all tested lines under drought, the two best performing lines had a better water use efficiency as well as better water use. This allowed higher biomass production in these two lines where higher biomass was directly related to higher efficiency of water use sustaining the photosynthetic machinery and also the ability to partition assimilates to plant growth and development. However, Blum (2005) postulated that effective use of water but not water-use efficiency should be the target for improvement of yield under drought. Since the two best performing lines effectively used the water in the growth medium under water-limiting conditions and also had better water-use efficiency, both parameters should be considered to contribute to better performance under drought. Therefore, both parameters should be determined to effectively select for drought-tolerant plants. In addition, for effective harvesting or assimilation of water from the growth media, abundance of the root system and also effective transport of absorbed water to above-ground plant parts are important for performance under drought (Banziger et al., 2000).

The best performing lines BAT 477 and BT_34-1-1 also had better SNF under both well-watered and drought conditions. SNF is a biological process demanding high energy and CO_2 assimilation as a carbon source for nodule growth and function. Sucrose synthesized by the plant is distributed to all plant part including the nodules. Sucrose synthase hydrolyzes sucrose in the nodule for providing the required carbon in nodules (Gordon et al., 1999). Previous studies have shown that sucrose synthase activity decreases in common bean and soybean after exposure to drought (Ramos et al., 1999). Furthermore, drought-tolerant bean lines had higher sucrose synthase activity than susceptible lines under drought (Ladrera et al., 2007). This suggests that the continuous supply of carbon to the nodules under water-limited condition is vital for better performance under drought enabling nodules to effectively provide SNF products to the plant.

Under drought, a positive and highly significant association was found between above-ground biomass and gas exchange parameters and SNF. In contrast a negative, non-significant relationship was found for both above-ground biomass and root biomass and nodule fresh mass. A positive association means that gas exchange parameters will determine above-ground biomass as well as SNF but both above-ground biomass and root biomass compete for assimilates with the nodules and there might be a competition for carbon between nodules and other plant parts.

Overall, results from this study greatly confirm the observations made with soybean regarding the importance of growth and gas exchange parameters as well as nitrogen fixing ability as performance markers to select superior performing bean lines for growth under drought. The existence of non-significant interaction for water treatment vs. bean lines for the plant

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performance traits measured for gas exchange, biomass (shoot and root) and SNF parameters suggests as these bean inbred lines performance were consistent across the two water regimes for these traits. This study allowed selecting the two bean lines BAT 477 and BT_34-1-1 as superior performing lines under drought when experiments were carried out under controlled environmental conditions in a phytotron. Therefore, a trait which would contribute for better accumulation to biomass under water-limited condition would be very important for enhanced drought performance and SNF ability. According to PCA analysis under water-limited condition, gas exchange parameters (A and G), growth parameters (leaf area and shoot as well as root mass) and nodule mass as well as SNF activity were the governing traits for bean lines performance variation. This indicates that, the relative growth of shoot vs. root were depend on the provision of nitrogen by SNF process by nodules and carbon by photosynthesis, at it has been also stated by Reynolds and Chen (1996) modeling study in this topic. Therefore, this overall result suggests use of these performance traits for drought tolerance screening in legumes improvement program especially under greenhouse studies. Further, testing of the performance of those lines under field conditions would be vital to obtain a better understanding of overall performance of these lines and to test the efficiency of performance characteristics as markers. Field trials also included the assessment of root architectural and morphological parameters.