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ABSTRACT

Drought causes considerable reduction of legume productivity and significantly threatens the food security, and this situation is expected to be aggravated due to climate change. In soybean and common bean, water resource capturing through plant root architectural plasticity and the role of symbiotic nitrogen fixation have not been investigated in greater detail yet. This study was therefore conducted to identify and apply useful morphological and physiological performance markers (traits) for selection of drought-tolerant common bean and soybean cultivars under both controlled phytotron and field conditions that might be applicable as markers in future legume breeding programs. In soybean, traits related to above ground performance, such as photosynthesis, biomasses, and stomatal conductance, were related to parameters for nitrogen acquisition in nodules. The ability to maintain vigorous shoot growth under drought-induced nitrogen limitation was identified as an important trait that can be used to select for improved drought tolerance. Further, experiments carried out growing different common bean inbred lines under controlled phytotron conditions revealed 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. As a further result, the strong association of symbiotic nitrogen fixation with CO_2 assimilation and stomatal conductance was also ascertained. In field experiments the effective use of water through enhanced lateral root development and maintaining the water status of the plant was found to be crucial for enhanced productivity under drought, with root morphology traits (root length, area and volume) as well as root architectural traits (first whorl angle, basal root number and adventitious root branching density) significantly related to seed yield. Measurement of these traits might be added to future bean varietal improvement programs. Further, a direct relationship between both water use efficiency (WUE) estimated using carbon isotope discrimination (CID) and nitrogen fixation (^{15}N abundance) with root morphological and architectural traits (root length, area and volume, basal root number, 1st as well as 2nd whorl angles) was identified. CID (WUE) and ^{15}N abundance (SNF ability) had a direct relationship with each other and also with productivity traits (seed yield and pod harvest index). Soybean field experiments verified the importance of root system architecture and morphology for providing drought tolerance with root architectural traits, tap and lateral roots (diameter and branching density) and morphological traits (root length, surface area and volume) contributing to better performance under drought. Moreover, the strong association of CID (WUE) with δ^{15}N (SNF), root traits as well as seed yield in soybean exposed to drought was ascertained. Findings suggested that higher performance in CID under drought stress may be due to higher CO_2 assimilation and better N_2 fixation resulting in better root system architecture and morphology of the drought-tolerant cultivar through maintenance of the water status of the plant for efficient biological activity. Overall the study has generated new knowledge about the use of physiological markers (traits) that can be used widely for legume evaluation under drought suitable for both phytotron and field studies.

THESIS COMPOSITION

Chapter 1 of this thesis provides a summary of the importance of grain legumes and effect of drought in common bean and soybean production. It also provides an overview of previous research on the effect of drought stress on legumes including shoot and root physiological performances as well as symbiotic nitrogen fixation ability. Further, the rationale, aim and objectives of the study are also presented at the end of the chapter. **Chapter 2** reports the results obtained for determining performance of different soybean cultivars under drought conditions in a growth chamber experiment. In particular, this chapter deals with the identification of easily measurable traits, such as gas exchange, plant growth and symbiotic nitrogen fixation, for plants grown under well-watered and drought conditions. **Chapter 3** reports about the physiological performance of different common bean inbred lines with varying degrees of drought tolerance grown in a phytotron under either adequate water supply or drought stress conditions. Performance traits measured under drought for soybean were also measured in order to identify performance traits more widely applicable for legumes. In **Chapter 4** the field performance of common bean inbred lines is reported. Especially this section considers the potential role of root architectural and morphological traits for identifying superior performing bean lines under drought conditions. The relationship of productivity traits with root system traits is also outlined for different nitrogen-fixing lines. **Chapter 5** reports the results obtained for evaluation of common bean lines for water use efficiency and symbiotic nitrogen fixation ability measured using stable carbon isotope discrimination (CID) and ^{15}N natural abundance respectively. Furthermore, the relationships of CID and ^{15}N natural abundance with plant productivity as well as morphological and architectural root traits are outlined in this chapter. **Chapter 6** presents the results obtained for determining the field performance of soybean cultivars. In particular, results of root morphological and architectural traits, plant productivity parameters, WUE (CID) and symbiotic nitrogen fixation (^{15}N natural abundance) performance of these soybean cultivars grown under drought and well-watered are reported. Finally, this chapter also deals with the results obtained on the association of especially CID and ^{15}N natural abundance with root and productivity performance traits. **Chapter 7** summarizes the findings and relevant information generated from this PhD study with the focus of how this study contributed to an advancement of the physiological understanding of the response of the shoot-root system of legumes for drought stress. It further highlights the importance of the use of root system architectural parameters, symbiotic nitrogen fixation traits together with other physiological traits for identification of drought tolerant legumes. Moreover, in this chapter a recommendation for application of performance traits for particular growth condition and legume type is provided. Finally, this chapter also outlines the possible future research activities which might help for using morpho-physiological performance traits for multiple stresses and for wider application in other tropical legumes. This is followed by the **reference** list of the citations used in this thesis and appendix.

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ABBREVIATIONS AND SYMBOLS

A	Assimilation
ABA	Abscisic acid
Adv.	Adventitious
ANOVA	Analysis of variance
ARA	Acetylene reduction assay
ATP	Adenosine tri-phosphate
ATP	Adenosine triphosphate
Bran.	Branching
C%	Carbon percentage
C:N	Carbon to nitrogen ration
C_2H_2	Acetylene
C_2H_4	Ethylene
CEC	Cation exchange capacity
Ci/Ca	Ratio of intracellular to ambient air CO_2 concentration
CIAT	International Center for Tropical Agriculture
CID	Carbon isotope discrimination
cm	Centimeter
CO_2	Carbon dioxide
CSA	Central Statistics Authority of Ethiopia
d	day
$d^{15}N/^{14}N$ ($\delta^{13}N$)	Delta of 15 nitrogen to 14 nitrogen isotope
df	Degree of freedom
DNA	Deoxyribonucleic acid
DTPA	Diethylene triamine pentaacetic acid
DW	Dry weight
EUW	Effective use of water
FABI	Forestry and Agricultural Biotechnology Institute
FAO	Food and Agriculture Organization of the United Nations

FAOSTAT	Statistics Division of the Food and Agriculture Organization of the United Nations
Fl	Flowering
FW	Fresh weight
G	Stomatal conductance
h	Hour
HI	Harvest index
IAEA	International Atomic Energy Agency
IITA	International Institute of Tropical Agriculture
IWUE	Instantaneous water use efficiency
KCl	Potassium chloride
LSmeans	Least Squares Means
$m^{-2}s^{-1}$	Meter square per second
MC	moisture content
mm	Millimeter
mmol	Millimol
MPa	mega Pascal
MPF	Mid pod filling
N	Nitrogen
na	Not applicable
Ndfa	Nitrogen drived from the aomosphare
NDPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NH_3	Ammonia
ns	Non significant
NUE	Nitrogen use efficiency
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PEPCase	Phosphoenolpyruvate carboxylase
PHI	Pod harvest index
QTL	Quantitative trait loci
r	Pearson’s correlation coefficient

RuBiSCO	Carboxylation of ribulose-1, 5-bisphosphate carboxylase oxygenase
RUBP	Ribulose-1-5- biphosphate
RuBPC-ase	Ribulose-1-5- biphosphate carboxalase
SCMR	SPAD chlorophyll meter readings
SE	Standard error
SEM	Standard error of the mean
SNF	Symbiotic nitrogen fixation
SWC	Soil water content
t	ton
URBC	Ukulima Root Biology Center
USD	United States Dollar
USDA	United States Department of Agriculture
WUE	Water use efficiency
WUE_{intr}	Intrinsic water use efficiency
WW	Well-watered
Y	Yield
$^{13}C/^{12}C$	Ratio of 13 Carbon to 12 Carbon
^{14}N	Nitrogen isotope with molecular mass of 14
^{15}N	Nitrogen isotope with molecular mass of 15
$^{15}N/^{14}N$	Ratio of 15 nitrogen to 14 nitrogen isotope
$\delta^{13}C$	Carbon isotope discrimination
θ_v	Volumetric water content
%Ndfa	Nitrogen derived from the atmosphere
µmol	Micromole
‰	Parts in thousands
^0C	Degree Celsius

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Importance of grain legumes

1.1.1 Production of common bean and soybean

Common bean production in Africa is estimated to represent 3,741,000 ha with about 25% of the total world production (11-12 million tons) (FAO, 2006). Figure 1.1 illustrates the areas of bean production in Sub-Saharan Africa (Wortmann et al., 1998). However, due to the development of new bean varieties and increased demand, the hectareage is expected to continue to increase. For example in Ethiopia, one of the biggest bean producers in Africa, bean production is currently extending to various regions of the country, becoming the second most important grain legume with production on 280,000 ha in the 2008/9 cropping year as shown in Figures 1.2 and 1.3 (CSA, 2010; Negash et al., 2011). Due to increasing demand and increased interventions in research and development in legume growing countries, the production of legumes has shown a remarkable increase in both hectareage and in production (Abate, 2012). For instance, in bean producing countries in Africa (e.g., in Eastern African countries such as Tanzania, Kenya and Ethiopia) the production of bean has reached up to 482, 390 and 267 thousand metric tons in the 2010 cropping year (TL II conference, 2011 country report, unpublished). This shows the increased demand and importance for beans and the need to solve production constraints, such as drought, low productivity and reduced profitability for the growers especially for moisture stress areas.

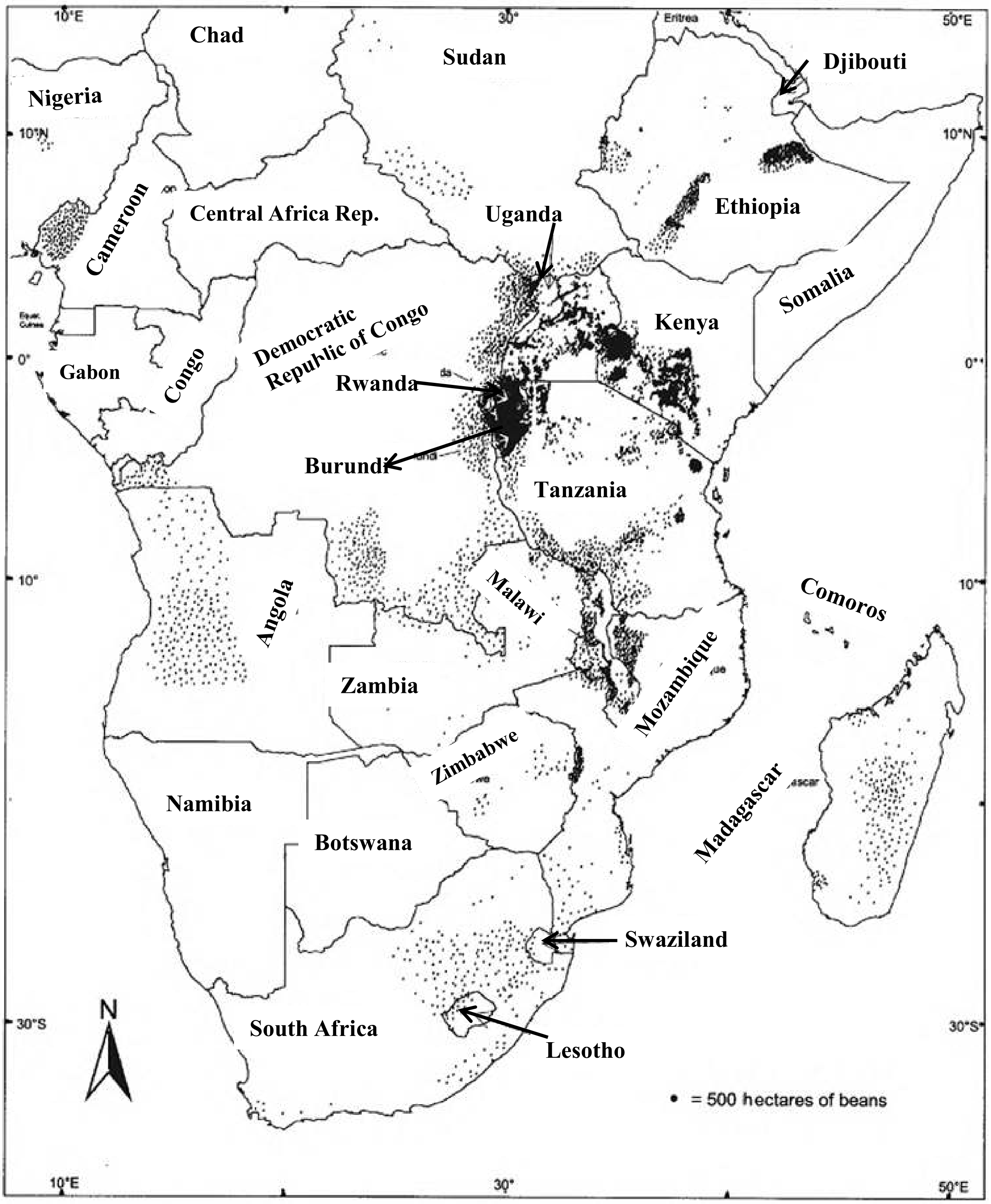


Figure 1.1: Distribution of bean production in sub-Saharan Africa (Source: Wortmann et al., 1998).

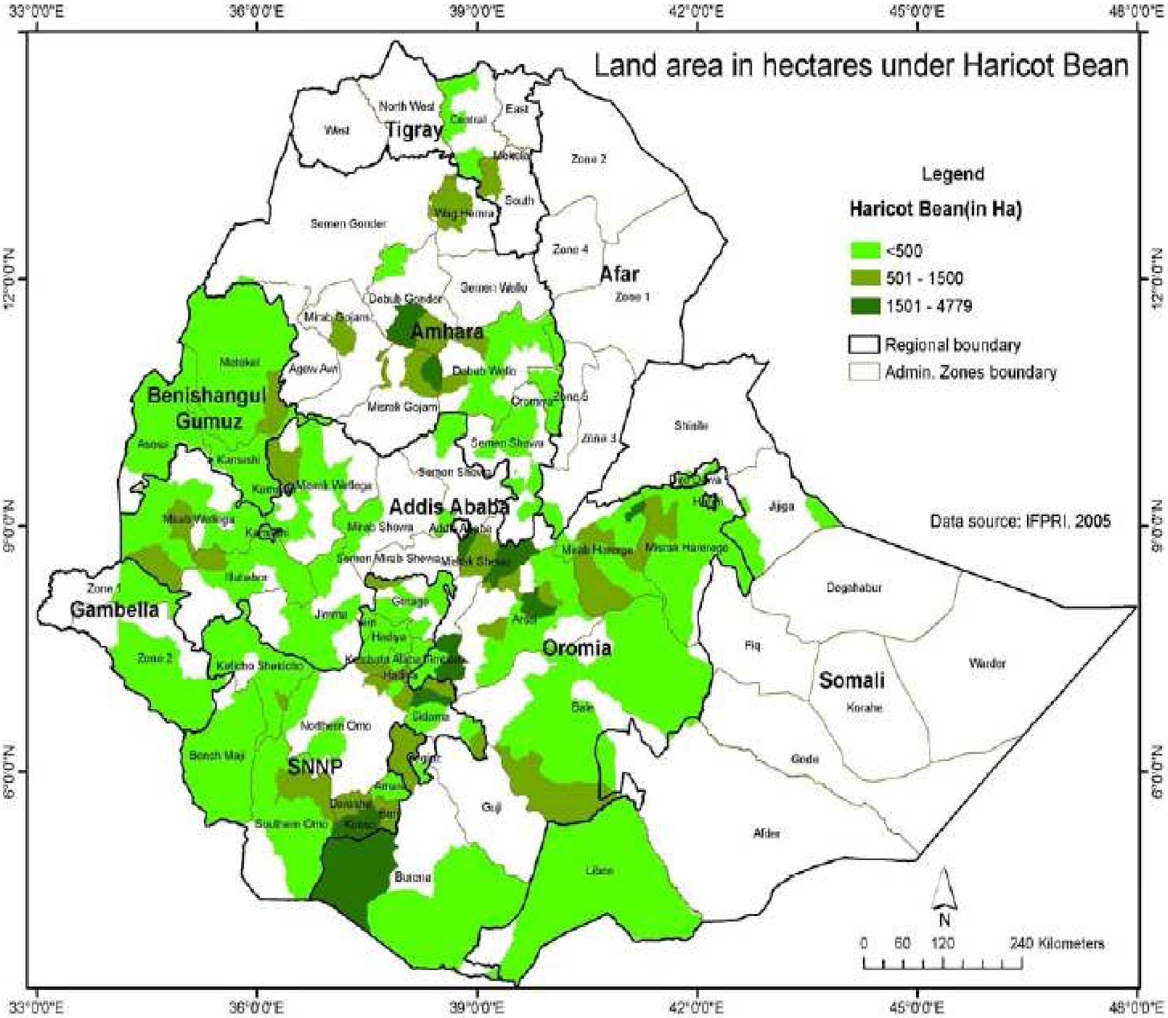


Figure 1.2: Geographic distribution of common bean production in Ethiopia for the year 2005 (Source: Negash et al., 2011).

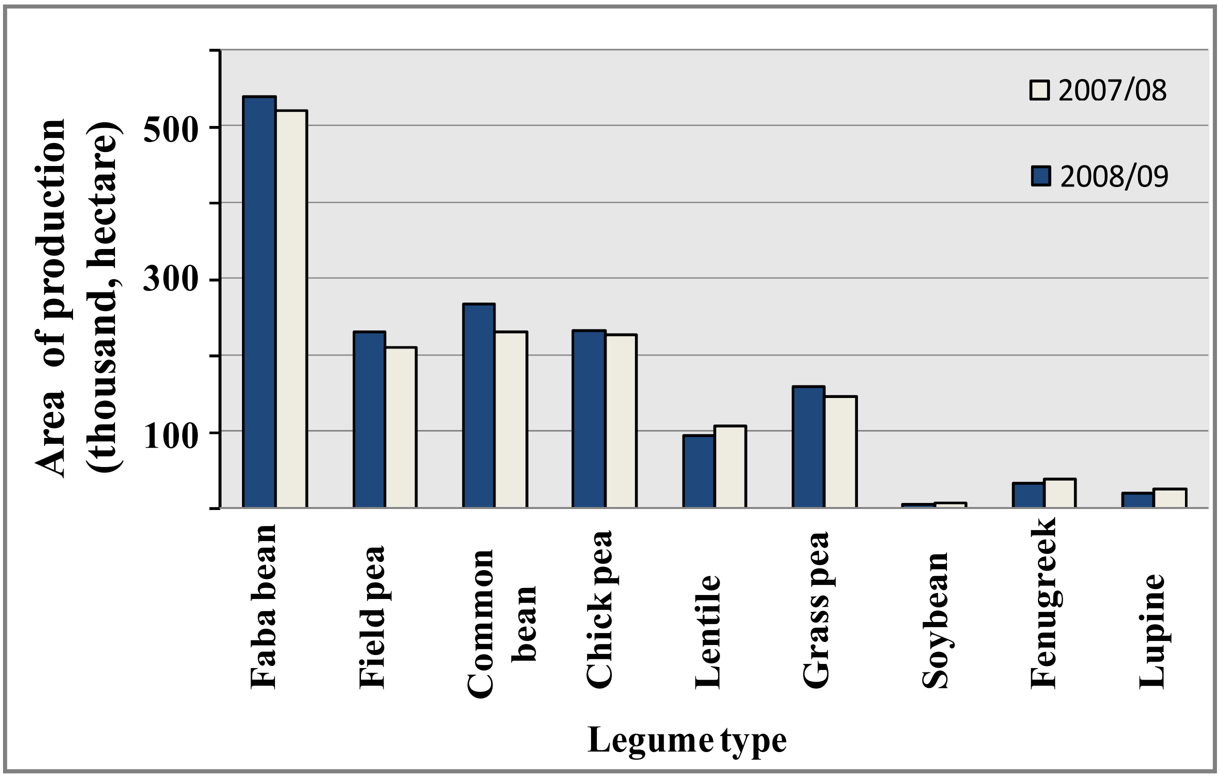


Figure 1.3: Estimated area of production of different legumes in Ethiopia for 2007/08 and 2008/09 cropping year (Source: CSA, 2010).

Soybean is produced globally on 94 million hectares of land with production of 223 million tons in 2006/08. Africa only produced 1.5 million tons which is about 1% of the world production (FAOSTAT, 2010). The total area of soybean cultivation in Africa is about 1.3 million ha with the three major soybean producers, Nigeria (625,667 ha), South Africa (199,323 ha) and Uganda (146,667 ha) (FAOSTAT, 2010). This accounts for about 80% of soybean production in Africa. Although the suitability map for soybean production in Africa shows a huge potential for the crop (Figure 1.4), and Africa reports and annual growth rate in soybean production of about 5%, this production increase is still not fast enough. If production growth continues according to this trend, the world-wide production will be 293 million tons in 2020 but with Africa still having a deficit of 196,000t (Abate, 2012). This is in spite of the growing demand for soybean for domestic processing of soybean meal and soybean oil in Africa. Particularly in Ethiopia soybean production has the potential to grow in most parts of the country and the production area coverage is expected to be 6826 ha (Tefera, 2011). This would rank Ethiopia as the 12th largest producer in Africa.

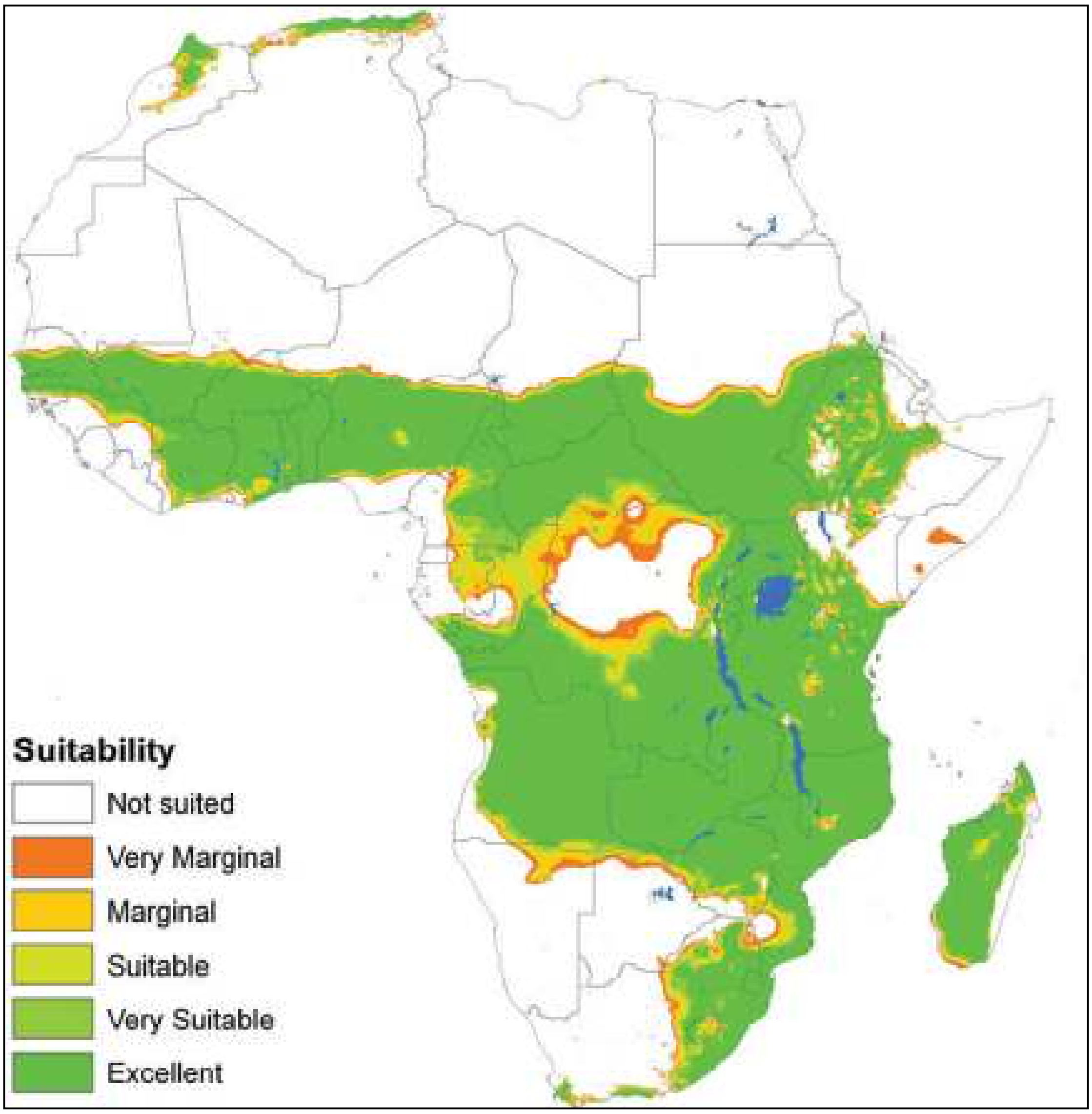


Figure 1.4: A map showing areas suitable for growing soybean in Africa (Source: IITA, 2009b).

1.1.2 Consumption and economic importance

Grain legumes, such as peas, common bean, and soybean are rich in protein, starch, fiber and other essential nutrients for human nutrition and animal feed (FAO, 2003). Among these legumes, common bean plays substantial role in direct consumption by small-holder farmers in sub-Saharan African and therefore has a significant role in improving human nutrition (CIAT, 2010). Especially in areas with a high population density such as Rwanda and Burundi, about 80% of the production is used for home consumption (Wortmann et al., 1998). In these areas per capita consumption of beans reaches up to 36 kg/year (FAO, 2001).

Soybean plays an important role in nutrition in Africa among subsistence farmers (IITA, 2009a). This is due to the increased demand of soy cooking oil, soy-fortified food and animal feed (especially for poultry). The production gap for soybean in Africa is met by imports. In 2008, Africa imported 3.6 million tons of soybean worth about 3.2×10^3 millions USD (Figure 1.5) (IITA, 2009a).

Common bean is a source of income for African economies by generating foreign exchange earnings and benefitting small-holder farmers. It has been estimated that Ethiopia’s export earnings from common bean is greater than 20 million USD per annum (Beebe et al., 2010) with an increasing trend during the last five years (Figure 1.6, CSA, 2010). In contrast, soybean is not exported. Generally, the demand for both common bean and soybean will continue to increase due to a higher world-wide demand for ready-made or processed canned foods.

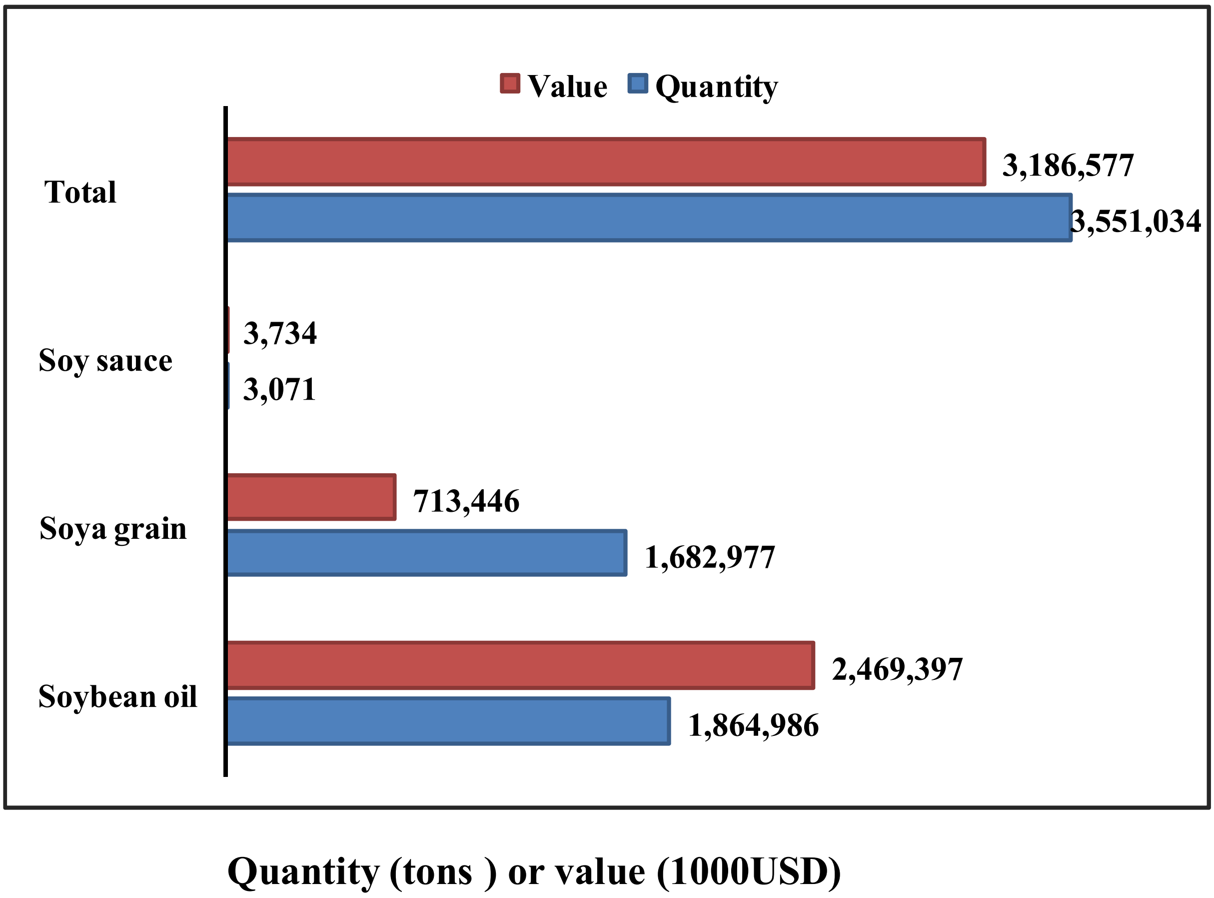


Figure 1.5: Import of soybean oil, grain and sauce into Africa for the year 2008 (Source: FAO, 2008).

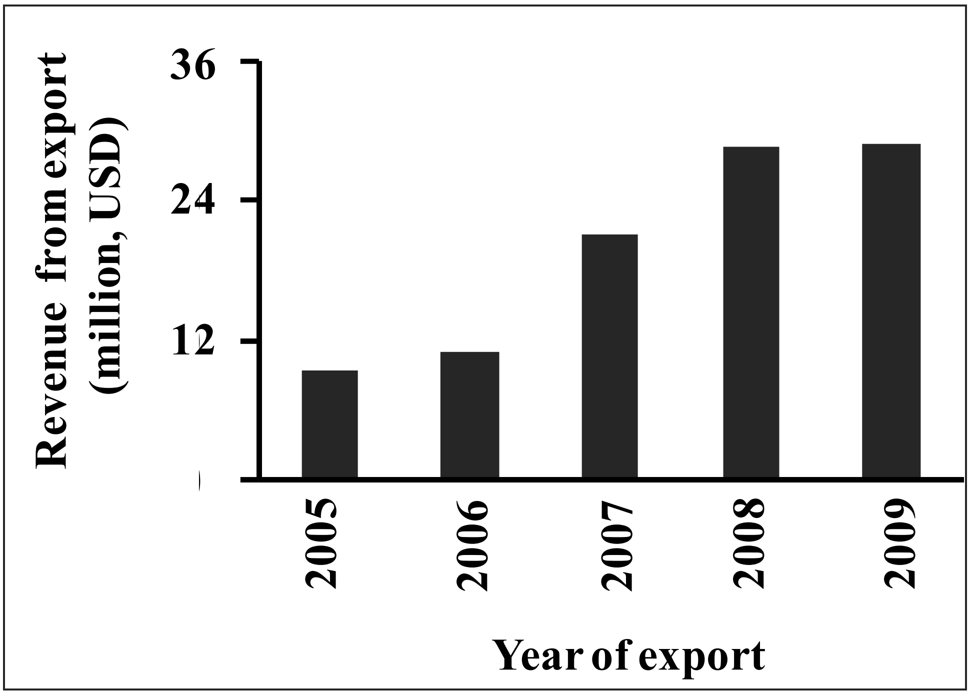


Figure 1.6: Ethiopia’s common bean export revenue for five consecutive years from 2005-2009 (Source: CSA, 2010).

1.1.3 Legumes and soil fertility

Legumes are also used as a natural nitrogen source in agriculture, particularly in Africa, through the presence of nitrogen-fixing bacteria in specialized organs (nodules) on the legume roots. Of the world's nitrogen demand, 60% ($3 \text{ x}10^9$ t N_2) is met by symbiotic nitrogen fixation (SNF), followed by chemical fertilizer (25%) (Zahran, 1999b). This shows the importance of grain legumes in farming systems world-wide.

The contribution of nitrogen fixation for the two legumes (soybean and common bean), which are the focus of this study, is reported as [49-450 kg/ha N (Herridge et al., 2008; Wani et al., 1995)] and [57 kg/ha N (Wani et al., 1995) to 100 kg/ha N (Hardarson et al., 1993)], respectively. Therefore, the use of these legumes provides cheap natural fertilizer, which is also favoured in the increasing organic market, where legumes play a great role in providing much of the needed nitrogen for other subsequent crops. Soil nutrient depletion is a prominent problem for subsistence farmers. According to Graham and Vance (2003), the average depletion for 37 countries was 22 kg N/ha per year. Moreover, this problem is further aggravated by the low fertilizer use in Africa (FAO, 2003). Therefore, production of grain legume has a vital role in the cropping system by providing the cheapest, and one of the most effective ways, to maintain sustainable yields in African agriculture.

1.2 **Legumes and drought stress**

1.2.1 Importance of drought stress

Drought stress is the primary challenge for crop production globally. Grain legume production is severely constrained by drought (Grzesiak et al., 1996; Sincik et al., 2008; Sinclair et al., 2007). Drought poses considerable reduction of plant productivity and significantly threatens food security (Boyer, 1982) especially in areas where the agricultural system is dependent on rainfall. About one-third of the world's agricultural land currently suffers from chronically inadequate water availability (Boyer, 1982; Flexas et al., 2006a; Ghannoum, 2009). This situation is predicted to become progressively worse and is a formidable challenge for future crop production (Chaves and Oliveira, 2004; Jury et al., 2007).

Moisture stress accounts for high crop loss in common bean and soybean production in Africa. For instance, in more than 60% of bean growing areas in the world drought restricts common bean production (White and Singh, 1991b; Wortmann et al., 1998). Currently this estimation might increase due to climate change (Chaves et al., 2003). Annual loss of common bean due to drought stress in moisture stress areas of Africa has been estimated to be about 781,000 t (Wortmann et al., 1998). In common bean, drought causes a loss (from 4 to 10 t/ha grain yield) for areas with rain fall between 300-450 mm. Complete crop loss occurs in areas with rainfall of less than 300 mm (Wortmann et al., 1998). Figure 1.7 shows the importance of moisture stress in bean growing areas in sub-Saharan Africa. Due to the expansion of the production area, and also the influence of climatic change, the extent of areas affected by moisture stress is expected to increase further. It has been estimated that 40% of current soybean producing areas, or those that are suitable for production in sub-Saharan Africa, mainly in Eastern and Southern Africa, is affected by water shortage (IITA, 2000). Research on drought tolerance is a major thematic area due to increased water stress that agricultural land may suffer in the future.

1.2.2 Research on drought stress

There are three common definition of drought (Whitmore, 2000; Wilhite and Glantz, 1985). Meteorological drought is defined as the prolong period when precipitation is significantly lower than the average value, which creates natural shortage of available water. Hydrological drought occurs when water reserves in the surface and sub-surface level (aquifers, lakes and reservoirs) decrease below the average value either due to shortage of rain or over consumption of water by humans. Agricultural drought occurs when the water supply (the precipitation and soil reserve) is unable to support crop production and restricts the expression of full genetic potential of the plant and causes reduction in crop yield (Turner and Brady, 1986). Based on time of drought prevalence and crop growth stage, agricultural drought can be further divided in to early season, mid-season, intermittent and late season (terminal) drought. However, terminal and intermittent drought are the most common type of drought affecting grain legumes production (Subbarao et al., 1995). These all suggests, the definition of drought is relative and differs based on the criteria and the concept of the water user (Whitmore, 2000).

Different types of drought adaptation mechanisms have been proposed. These are drought escape, drought avoidance and drought tolerance. Drought escape is defined as the ability of the plant to complete its life span (flower, set pod, fill grain and mature) before the onset of water shortage (Beebe et al., 2010; Turner et al., 2001). In areas experiencing a short duration of rain fall or predicted to have terminal drought, varieties that escape drought perform better (Siddique et al., 1999; Thomson et al., 1997). Selection of early maturing grain legumes can have, however, undesirable impact on grain yield. Therefore, a variety with a flexible maturity might be more beneficial (Turner et al., 2001).

Drought avoidance is defined as the ability of the plant to sustain high tissue water content and potential under water limiting conditions. Drought avoidance strategies include reducing water loss by minimizing the expansion of leaf area and reducing stomatal conductance or by leaf movement / rolling. Further, better water absorption by extending root depth or increased root density and hydraulic conductance are also important mechanisms for drought avoidance (Manavalan et al., 2009; Morgan, 1992). Drought tolerance is defined as capacity of the plant to resist drought, i.e. decreased cellular water content or water potential. This may be achieved through osmotic adjustment which decreases cellular concentrations of osmolytes and increases water movement into the cell and tissue. This maintains turgor. Adjustment can be achieved through synthesis of compounds such as amino acids (proline) and sugars or other compatible solutes in the tissue or transported into it (Amede and Schubert, 2003; Jongdee et al., 2002; Nguyen et al., 1997). Such adaptations delay metabolic damage and leaf senescence and improves the transport of assimilate to the grain (Leport et al., 1999), thereby improving root development and water absorption (Morgan and Condon, 1986) and stomatal conductance and CO_2 assimilation. However, greater stomatal conductance will lead to faster water loss and so off-set the advantages of the adaptations.

Even though many researchers have been involved in drought related studies, the success is still very limited. Research has focused on the identification of common bean cultivars adapting to drought stress and promising drought-tolerant common bean lines as parental materials to create recombinant inbred lines parents (BAT 477, G21212, ICA Quimbaya and SEA 5) (Beebe et al., 2010). Although drought is one of the main production constraints for soybean in Africa, progress in developing drought-tolerant varieties is still slow with only a few promising soybean lines identified (Tefera, 2011).

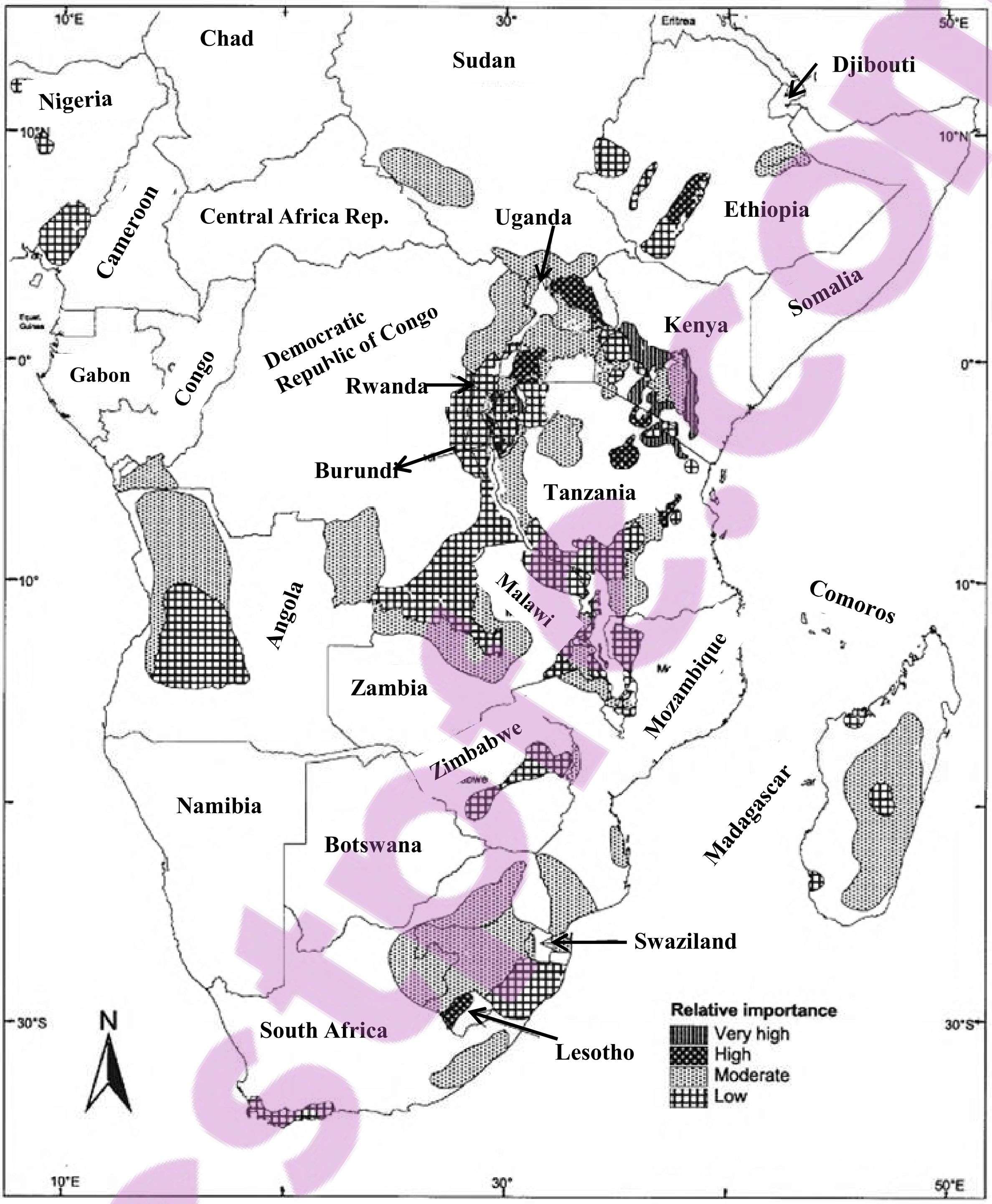


Figure 1.7: Relative importance of soil moisture deficits in bean production areas of sub-Saharan Africa (Source: Wortmann et al., 1998).

Although past studies contributed to an understanding of drought stress tolerance, most of the studies were focused only on a specific plant performance trait. Moreover, the contribution of roots and the effect of water stress on symbiotic nitrogen fixation (SNF) have been less studied. O'Toole and De Datta (1986) stated that "drought is a syndrome" due to its complex behaviour and its uncertainty of prevalence, period and intensity of its persistence. Thus, success in developing a better performing legume under drought necessitates a comprehensive understanding of the physiological and morphological characteristics of above ground (shoot) and below ground (root) plant parts for drought tolerance as well as the symbiotic nitrogen fixing ability for the identification of main adaptation traits. This might help to develop selection criteria to support varietal improvement for devising effective breeding strategies for drought tolerance.

1.2.3 Physiological effect of drought on legume performance

White and Singh (1991b) defined drought in bean as, "the insufficiency of water availability during the growth cycle of the crop which limits the maximum expression of genetic potential". Therefore, developing better performing legumes under drought, exploiting the genetic potential of the existing germplasm and also breeding for enhanced tolerance, remains an important task for plant scientists.

1.2.3.1 Leaf water potential

Water deprivation leads to loss of water from the plant and changes the plant water status with a decline in the stomatal conductance and transpiration (Ribas-Carbo et al., 2005). Since leaf water potential is a good indicator of the plant water status (Turner, 1982), it is an important and dependable performance indicator in drought studies, and maintenance of the leaf water status is a key indicator for dehydration avoidance (Jongdee et al., 2002; Siddique et al., 2000). Reducing water loss through stomatal closure, rolling or abscission of the leaf, and increased plant water up-take through enhanced root development are mechanisms playing a role in maintaining the leaf water status (Jongdee et al., 2002). However, maintenance of leaf turgor through accumulation of solute have also been identified to be an adaptation strategy for maintaining the water status in legumes (Amede and Schubert, 2003). Plants which maintain the water status (high water potential) have been found to be productive under stress condition with low reproductive abortion (Jongdee et al., 2002; Pantuwan et al., 2002). Therefore, as leaf water status determines the gas exchange of the plant, it should be essential to use leaf water status as a selection indicator for drought.

1.2.3.2 Photosynthesis

The gas exchange of the plants (as reflected in CO_2 assimilation, transpiration and stomatal conductance) is the principal plant process responsible for plant biomass production and for plant adaptation to a change in environment (Lawlor and Tezara, 2009a). Stomatal opening, which controls gas exchange, is a sensitive indicator for drought stress. In common bean, and most other legumes, stomatal closure for reducing the water loss is one of the adaptation strategies to drought (Miyashita et al., 2005). However, stomatal closure also results in reduced CO_2 movement for carboxylation within the chloroplast. This can be a major cause of drought-induced decreases in CO_2 assimilation capacity, particularly in C_3 plants (Chaves and Oliveira, 2004; Flexas et al., 2006b; Warren, 2008), and causes reduced leaf expansion and plant biomass production (Chaves et al., 2003; Lawlor and Tezara, 2009a). Furthermore, during the process of photosynthesis, photochemical and biochemical activities occur in the leaf and these activities are seriously affected by drought stress (Chaves et al., 2002). Since CO_2 assimilation is the major factor in the plant's supply of carbon and ATP, and is susceptible to drought (Lawlor, 2002a; Parry et al., 2002), measurement of CO_2 assimilation will continue to be a major target in drought stress studies. Further, since the adaptive strategies of legumes for water deficit are either stomatal closure or heliotropic leaf movement (Pastenes et al., 2005), legumes which can sustain stomatal conductance or have enhanced CO_2 assimilation per unit of stomatal opening will be a target for future plant improvement programs. Also, complementing existing information on gas exchange performance and understanding the relationship with other performance traits will be vital for using these traits for evaluation of legumes in future varietal improvement program. Existence of genetic variability for gas exchange performance in common bean (Comstock and Ehleringer, 1993; Gebeyehu, 2006; Mencuccini et al., 2000) and soybean (Fenta et al., 2011; Flexas et al., 2006a; Liu et al., 2005) has already been reported

1.2.3.3 Water use efficiency

Water use efficiency has several definitions and varies depending on scale, e.g., plant leaf or whole plant or time, e.g., short-time scale of minutes or longer-term up whole plant growth season (Bacon, 2004). For the whole plant over the growth season, water use efficiency is defined as the ratio between production of biomass, shoot biomass or harvested yield and total evapo-transpiration or plant transpiration (Chaves and Oliveira, 2004; Connor et al., 1992). For the plant leaf, WUE is defined as the ratio between instantaneous net CO_2 assimilation rate (A) and transpiration (E) (A/E). Since A/E largely depends on vapour pressure deficit, the ration between CO_2 assimilation and stomatal conductance (G) (A/G), which is termed intrinsic water use efficiency, is usually used as a normalized value when compared to instantaneous water use efficiency (Chaves and Oliveira, 2004; Farquhar et al., 1989; Soares-Cordeiro et al., 2009b). Intrinsic water use efficiency provides a direct measure of activity of the photosynthetic system normalized to constant stomatal conductance. The use of CID (carbon isotope discrimination) also provides a measure of intrinsic water use efficiency and primarily measures the ratio of A/G) (Bacon, 2004).

Using CID is simple and rapidly estimates WUE over time (Farquhar et al., 1982). This concept was further supported by CID research work in higher plants by Evans et al. (1986), who established as CID reveals CO_2 assimilation of the weighted average throughout the growth period of the plant. Furthermore, in the field experiments application of WUE needs measurement of whole plant or field level crop water use, apart from its tediousness, it is labour and time consuming. The innovation of CID as a heritable trait for understanding the gas exchange mechanism in plants at the whole plant level as an estimate of WUE simplified the measurement of WUE at the field level (Farquhar et al., 1989; Hall et al., 1998). Additionally, the correlation of lower CID value measured at the leaf as well as grain with higher WUE in most studies (Blum, 2011) further suggest the importance of the use of CID for WUE measurements.For this reason, CID measurement has become one of the selection traits for drought screening in maize and wheat at CIMMYT (Pask et al., 2012). So far, CID has not been widely applied in a legume improvement program.

Traits that serve to conserve water (conservative traits) include low stomatal conductance, low leaf growth rate, or deep root systems provides better water use efficiency. Research often focused on the use of instantaneous water use efficiency (IWUE) values as a physiological marker for drought tolerance. Higher IWUE values indicate improved tolerance to drought since varieties with high IWUE values are better able to assimilate carbon at low stomatal conductance and hence attain a greater yield using less water. Confirmation of a direct relationship of WUE with A/G, C_i/C_a (ratio of intracellular to ambient air CO_2 concentration) and with carbon isotope discrimination ($\delta^{13}C$ measurement) provides a tool for WUE evaluation (Farquhar and Richards, 1984; Farquhar et al., 1982; O'Leary, 1988). The biochemical basis for $\delta^{13}C$ measurement in C_3 plant is due to the inherent discrimination of ^{13}C by ribulose-1-5-biphosphate carboxylase (RuBPC-ase) in favour of $^{12}CO_2$ (by a factor of ~27‰), because of lower reactivity of ^{13}C (Farquhar et al., 1982; Farquhar et al., 1989). CID ($^{13}C/^{12}C$) has been applied for WUE efficiency evaluation in several crops (Farquhar and Richards, 1984; Farquhar et al., 1982; Martin and Thorstenson, 1988; Rytter, 2005; Saranga et al., 1998). This allowed evaluation of a large number of germplasm for WUE under controlled and field experiments measuring the amount of water consumed by the plant (Rytter, 2005).

Due to the existence of a negative association of CID with WUE in several different C_3 plants (Farquhar et al., 1989) including wheat (Condon et al., 1990; Farquhar and Richards, 1984), alfalfa (Johnson and Rumbaugh, 1995; Johnson and Tieszen, 1994), cool season grass (Johnson and Asay, 1993) and barley (Anyia et al., 2005), low CID has been used as a selection criterion for enhanced WUE (Shaheen and Hood-Nowotny, 2005). Moreover, the negative association of CID with plant biomass and seed yield for drought tolerant cereals (Anyia et al., 2005; Condon et al., 2002; Shaheen and Hood-Nowotny, 2005; Teulat et al., 2001) supports the importance CID as a selection criterion for enhanced WUE.

Variation for WUE has been observed in soybean (Hufstetler et al., 2007) and in common bean (Ehleringer, 1990; Gebeyehu, 2006) regarding the amount of dry matter produced per given amount of water and CID. More importantly, the moderate to high heritability of CID found in cowpea (Menéndez and Hall, 1996), common bean (Ehleringer, 1988), wheat (Araus et al., 1998) and cool season grasses (Johnson et al., 1990) provides additional support of the use of CID for water stress evaluation. However, breeding plants for high WUE under drought might actually result in low-yielding plants (Blum, 2011) when WUE is not associated with productivity traits (Menéndez and Hall, 1996). Therefore, the usefulness of IWUE or CID as a selection parameter depends on its contribution to productivity and association to productive traits under drought (Menéndez and Hall, 1996).

1.2.3.4 *Plant growth, biomass and productivity*

Of the wide range of possible morphological characteristics that can be used in the selection of legume varieties for enhanced drought tolerance, shoot parameters are generally considered to be the easiest to assess under field conditions. Shoot markers remain major targets in breeding programs, particularly in developing countries, where variations in shoot morphology are often determined subjectively under field or glasshouse conditions (Manavalan et al., 2009). Often this involves monitoring leaf area (Mohamed et al., 2002), dry matter yield per plant (Mohamed et al., 2002; Udensi et al., 2010), harvest index, and finally grain yield (Gebeyehu, 2006; Muñoz-Perea et al., 2007).

Leaf shading and reduced leaf elongation (Acosta Gallegos, 1988) through inhibition of cell division and expansion (Zhu, 2001), adjustment in days to maturity and rapid biomass accumulation (Siddique et al., 2001) have been found to be adaptive strategies under drought stress. Reducing biomass or leaf area to minimize stomatal opening and reducing transpiration rate is important for terminal drought but these characteristics might be less favourable for a longer drought period resulting in less plant biomass and seed yield (Blum, 2011). Slow plant growth for better assimilate partitioning and production of protective compounds (Zhu, 2002) as well as enhanced root development for maintaining plant water status and cellular activity (Chaves et al., 2003) have also been found to be adaptation traits for drought stress that contribute to more harvestable yield. Therefore, drought avoidance through maintaining the plant function might be a target for selection for breeders to improve plant productivity under stress condition (Chaves et al., 2003).

Improving plant productivity under drought condition requires selection for a higher biomass accumulating genotype (Lopes et al., 2011), since biomass and grain yield have a strong positive association, especially in grain legumes (Ramirez-Vallejo and Kelly, 1998; Shenkut and Brick, 2003). Furthermore, previous studies also ascertained the high heritability of plant biomass (Shenkut and Brick, 2003). As in other crops, legume biomass and productivity has been found severely restricted by drought stress and these traits can be used as selection markers for performance under drought (Beebe et al., 2010). Plant productivity is also a function of the amount of interception of photosynthetically active radiation (PAR). Zhu et al.(2010) estimated the efficiency of PAR interception and the effectiveness of converting assimilate into biomass and grain yield. Plant performance is further associated with effective use of water through better root development as a drought avoidance mechanism (Blum, 2011). Since higher root development can be a trade-off for shoot as well as grain production, the cultivar which maintains a better root: shoot ratio has an advantage. However, this is highly related to the nitrogen use as well as photosynthetic efficiency of the plant (ÅGren and Franklin, 2003; ÅGren and Ingestad, 1987). In addition, understanding the relationship of these productivity traits with other performance traits will also be vital to determine the contribution of different parameters to yield.

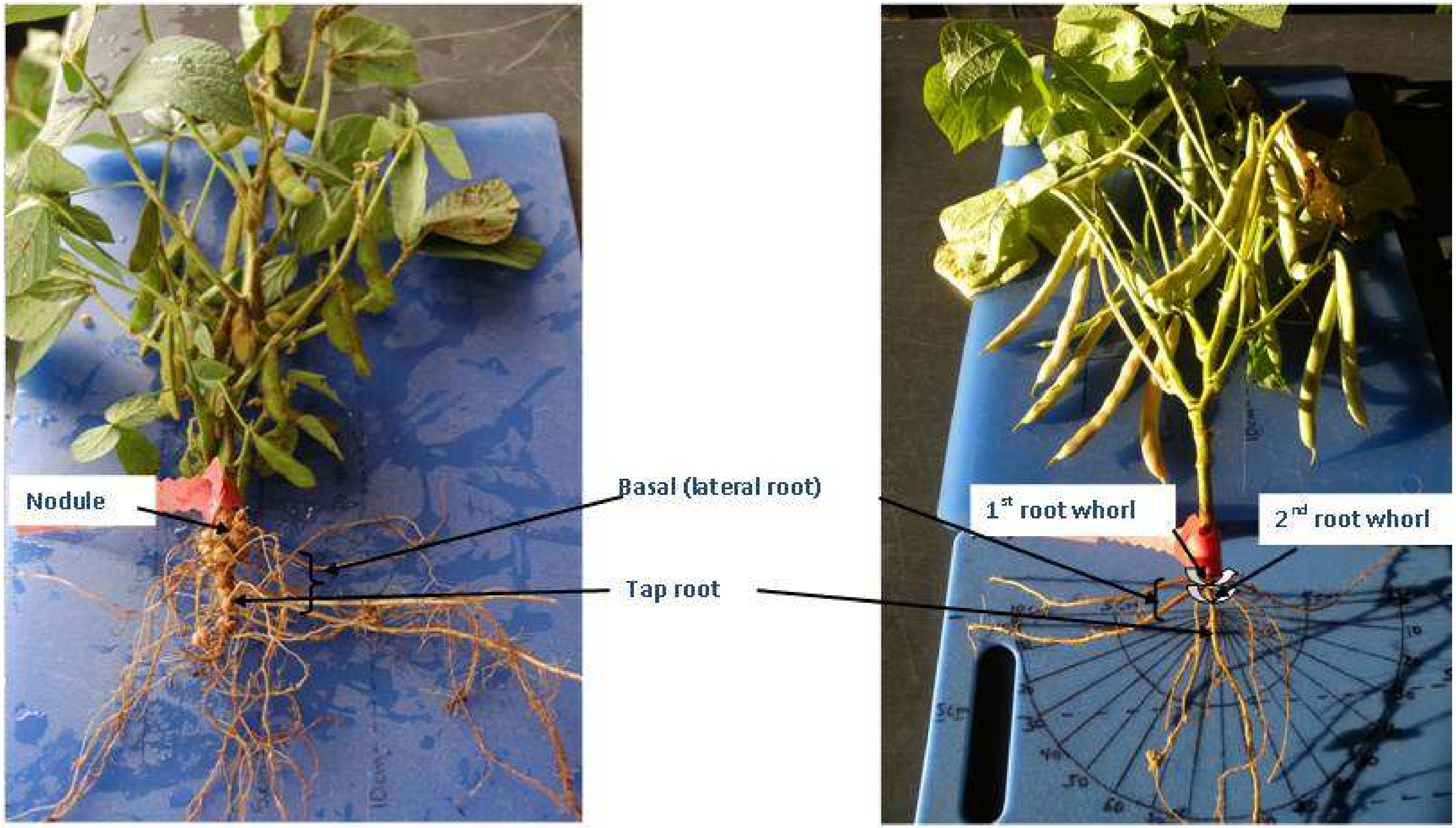
1.3 Effect of drought on roots

1.3.1 Root system of legumes

The root system is the principal plant organ which provides absorption of water and essential nutrients from the soil (Malamy, 2005). However, since these soil resources are limited and differ depending up on the soil type and environmental factors, the survival of the plant depends mainly on the inherent root morphology and architecture along with the modulation of the root structure in response to the external stimuli (Fitter, 1987). As common bean and soybean are mainly grown in water-limited tropical and sub-tropical areas in Africa, root traits play a fundamental role for adaptation as well as for enhanced productivity (CIAT, 2007; IITA, 2009a).

In general, root morphology refers to the external features of root. These include characteristics such as root length, diameter, area, volume and number of root tips (Lynch, 1995; Regent Instruments Inc., 2011). Root architecture is the spatial configuration (three dimensional structures) of the root system at a point in time, considering the different parts of the root system (tap root, lateral roots and root hairs) (Lynch, 1995; Lynch, 2007; Osmont et al., 2007). The root system in flowering plants is classified into two types. The allorhizic root system is the first type and is commonly found in the dicot plants (Osmont et al., 2007). Soybean and common bean also have this typical root system. In the allorhizic root system there are two main types of roots, the primary root (tap root) and lateral (basal) roots (Figure 1.8). The first root that emerges from the embryo is the tap root. The other roots, which emerge from the tap root and which can produce the higher order roots (branches), are the lateral (basal) roots (Esau, 1965; Larcher, 2003). The basal roots in common bean emerge in a circular pattern at the point of initiation of the tap root and this pattern is called root whorl. The number of whorls might vary across different genotypes mostly from 1 to 3. The lateral roots are considered to be basal when it originated from primary root (radicle) at early germination stage. The sequence of the whorls is from the top to the base of the tap root (Figure 1.8). The allorhizic root system has also adventitious roots. These roots emerge above the root whorls (basal roots) from the stem or hypocotyls. The second type of root system is the homorhizic root system which is common to monocot plants (Esau, 1965; Larcher, 2003).

The configuration of the root organ is quite stable and controlled by inherent genetic factors of plant species. However, the amount, placement and direction of root growth vary, even within the same species (Malamy, 2005). The extent of developmental plasticity of the root system for these diverse characteristics of the root in the soil environment is controlled by hormones responding to external stimuli (Bao et al., 2004). In particular, auxin has a major role in controlling the root development in plants (Lucas et al., 2008). The emergence as well as the growth and development of lateral roots are both controlled by auxin (Casimiro et al., 2001; De Smet et al., 2007). In legumes, abscisic acid (ABA) has a role for lateral root development (Liang et al., 2007). Under drought condition, both ABA and water stress synergistically enhance the lateral root development and contribute to drought performance (Xiong et al., 2006). However, enhanced ABA activity during drought stress has a negative impact on the legumes nodule number.



Soybean

Common bean

Figure 1.8: Soybean and common bean plants with major root types.

1.3.2 Root architecture and morphology

Advances towards improvement for drought tolerance have mainly been based on the evaluation of above ground (shoot) traits. The complexity of drought stress and the rather little activity in root research has limited major advances in drought stress tolerance in legumes (Blum, 2005). This might be due to the difficulty in measuring the below ground root system architecture, such that most plant scientist are reluctant to work with roots (Nielsen et al., 1997).

Drought affects the development of root architecture due to its role as an initial sensor organ to water deficit. Plants generally modify their root architecture and increase total root absorption surface area by new lateral root formation and elongation as a mechanism for avoiding drought stress (Osmont et al., 2007). Unlike other dehydration avoidance strategies (stomatal closure, leaf rolling or abscission), dehydration avoidance through improved root development sustains productivity of plants by maintaining the plant water status and photosynthetic assimilation (Lopes et al., 2011). Previous research has also shown the importance of deep rooting for better performance under drought in beans (White and Castillo, 1991), wheat (Reynolds et al., 2007) and rice (Li et al., 2005). Therefore, better understanding of root traits would be vital for improving the legume selection strategy to sustain productivity under water-limited conditions. Other research groups have also demonstrated the importance of nitrate for root development and nodulation (Gresshoff, 2010). Therefore, this suggests the requirement of not only searching for better root development under drought, but also performance under nitrate starvation or SNF ability.

Research has further shown the existence of genetic variability in common bean for phosphorous stress and in particular for basal and tap root development (Beebe et al., 2006) as well as for root density and branching (Lynch, 2007). Common bean also expresses genetic variability in root growth in response to water deficit with deep rooting ability or enhanced root mass (Sponchiado et al., 1989; White and Castillo, 1991). In soybean, root architectural (root diameter and length) (Ao et al., 2010; Zhao et al., 2004) and morphological traits (root volume, area and length) (Ao et al., 2010) have also been investigated as an adaptive strategy for drought stress.

Despite the existence of variability in legumes for root traits, the use of these traits in a plant improvement program still needs to be confirmed as a valuable contribution to productivity. The contribution of root traits to plant growth and productivity has been investigated in common bean (Sponchiado et al., 1989) and chickpea (Kashiwagi et al., 2006).There is a relation between root features and plant productivity in terms of yield, water use or nutrient capture (Steele et al., 2007). Evidence of a relationship between root architecture and plant productivity, water use or nitrogen use efficiency (Garnett et al., 2009) would also provide insights into the relationship of root system architecture with symbiotic nitrogen fixation (SNF) traits. This was therefore a major focus of this PhD study.

The production of plant biomass and grain yield under drought also depends on the maximum moisture capture to satisfy the transpiration demand. This efficiency is termed “effective use of water (EUW)” (Blum, 2011). Oxygen isotope enrichment (δO_2) has been found to be a good proxy for the transpiration rate providing information about the amount of water supplied by root development to meet the evaporative demand (Sheshshayee et al., 2005). Moreover, the existence of a positive association of root mass with δO_2 (Elazab et al., 2012) further indicates that root traits are useful indicators for EUW. Also, the adverse effect of drought in plants is effectively avoided by changing assimilate allocation to allow for better root development before the onset of the adverse effect on plant development (Lopes et al., 2011). Further, root development and nodulation are highly dependent on the soil moisture availability (Garside et al., 1992).

1.3.3 Effect of drought on nodules

Legumes are highly important in supplying nitrogen through symbiotic nitrogen fixation (SNF) (Herridge et al., 2008). Legumes nodules are formed due to the symbiotic interaction of the legume and bacteria (Serraj et al., 1999) in common bean (*Rhizobium leguminosarum* bv. *phaseoli*) and soybean (*Bradyrhizobium japonicum*). Both these tropical legumes produce a determinate type of nodule. Photosynthetic assimilates and other metabolites are transported to the nodule through diffusion from the phloem in the nodule cortex. The SNF products, usually ureides (allantoin and allantonic acid), are transported to the shoot via the xylem (Schubert et al., 1995). However, nodule infection to establish a symbiosis as well as the bacterial activity are severely restricted by drought (Kirda et al., 1989). Generally, drought reduces the quantity of rhizobial bacteria in the soil, and their development and infection ability. This affects the formation of nodules and synthesis of leghemoglobin. Finally, the nodule life span and SNF are severely affected by drought (Hungria and Vargas, 2000; Venkateswarlu et al., 1990).

Reducing N_2 by the nitrogenase in the SNF process consumes high energy derived from photosynthesis as shown in the equation below (Serraj et al., 1999):

$$N_2 + 8H^+ + 16ATP + 8e^- \longrightarrow 2NH_3 + H_2 + 16ADP + Pi$$

Further, according to (Rainbird et al., 1984) to sustain nitrogenase for nodule growth and for N transport, a total of 12.2 g of carbohydrate per g of N fixed is used by soybean nodules. SNF is therefore an energy demanding process. Any process which limits plant photosynthesis for supplying carbohydrate also affects SNF. Under water deficit, reduced carbon assimilates further affects SNF efficiency in legumes. Also, the high energy demand of the nodule requires a high nodule flux of oxygen for respiration. Therefore, maintaining and regulating the demand and supply of oxygen by the nodules during drought is a challenge. Nitrogenase is inhibited by O_2 (Minchin, 1997). To ensure proper function of root nodules, leghemoglobin in root nodules carries oxygen and also buffers free oxygen in the cytoplasm of the nodule cells. Since leghemoglobin stores oxygen for optimal nodule respiration and transport to respiring symbiotic bacteria for a few seconds (Denison and Harter, 1995), a continuous supply of oxygen for functioning is required. A decline in oxygen permeability due to the creation of oxygen diffusion barrier results in reduced SNF activity (Denison, 1998). Therefore, any decline in the oxygen flux caused by the drought as a plant response reduces SNF activity of the legumes (Arrese-Igor et al., 2011).

1.3.4 SNF and plant biomass and productivity

SNF, measured as the amount of N accumulation, is sensitive to drought (Serraj et al., 1997; Sinclair, 1986). In field experiments, drought reduced SNF (N accumulation in the shoot) in soybean by 56% and biomass by 42% relative to well-watered controls (King and Purcell, 2006). Drought affects biomass and SNF ability in most legumes including common bean (Castellanos et al., 1996b) and mung bean (Thomas et al., 2004) with SNF more affected than biomass. However, decrease in N-accumulation is not only due to the decline in SNF ability, but is also a consequence of a decreased biomass amount (Streeter, 2003). This suggests a significant role of SNF in maintaining plant biomass and *vice versa*. Further, a strong association between seed yield and different SNF performance traits, such as ^{15}N natural abundance, nodules mass and number has been found (Pazdernik et al., 1997; Ronis et al., 1985). Also, genetic variability associated with various degrees of sensitivity of SNF to drought has been found for soybean (King and Purcell, 2006; Pazdernik et al., 1996) and common bean (Castellanos et al., 1996b; Sinclair and Serraj, 1995).

1.3.5 Methods of SNF measurement

1.3.5.1 ^{15}N analysis

Several methods have been used for quantifying N_2 fixation ability of legumes. This includes ^{15}N natural abundance and nitrogenase activity, or acetylene reduction assay (ARA) techniques. In nature, there are two principal isotopes of nitrogen: ^{14}N and ^{15}N. Even though the chemical characteristics of these N isotopes are similar, they have small quantitative differences due to their difference in mass and activation energy. In nature, the lighter isotope ^{14}N is highly abundant. The isotope ^{15}N represents 0.36663% of atmospheric nitrogen and this concentration is termed "natural abundance" (Högberg, 1997). Therefore, if the ^{15}N concentration in the atmosphere differs from the plant available soil N, and these values are known, N_2 fixation can be calculated on the basis of ^{15}N analysis of a N-fixing legume and a non-fixing plant. This difference is expressed as $\delta^{15}N$ parts in thousands (‰) relative to ^{15}N of the atmosphere (Shearer and Kohl, 1986). This method is used for SNF performance measurement in common bean (Castellanos et al., 1996b), soybean (King and Purcell, 2006) and also mung bean (Thomas et al., 2004). Although this SNF measurement method needs specialized equipment (mass spectrometer) and the cost associated with this measurement can be expensive, the technique is considered to be a direct measurement of N_2 fixation (Peoples et al., 1989).

1.3.5.2 <u>Nitrogenase activity assay (ARA)</u>

The principle behind the acetylene reduction assay is that nitrogenase, found in the N_2-fixing bacteria, reduces N_2 to ammonia (NH_3) in legume nodules. This enzyme is also able to reduce acetylene (C_2H_2) to ethylene (C_2H_4). Therefore, C_2H_4 can be use as an alternative substrate of nitrogenase (Hardy et al., 1973). By placing nodule roots, either detached or with the plant root system, in an air tight vessel and incubating with C_2H_2, the amount of C_2H_4 produced over a certain period is quantified using a gas chromatograph. This can be expressed in the following equations:

$$N_2 + 8H^+ + 8e^- \xrightarrow[\text{nitrogenase}]{} 2\ NH_3 + H_2$$

$$4C_2H_2 + 8H^+ + 8e^- \xrightarrow[\text{nitrogenase}]{} 4\ C_2H_4$$

This method is considered to be an indirect method of assessing SNF by determining enzyme activity based on the electron flux through nitrogenase. The method is a simple, rapid and very sensitive analytical method for detecting nitrogenase activity. However, variation of enzyme activity in intact and detached material necessitates taking several measurements (Peoples and Herridge, 1990). This method has been used for SNF measurement in faba bean (Plies et al., 1995) and also ten other legumes including common bean, soybean (Sinclair and Serraj, 1995) and chickpea (Thavarajah and Ball, 2006).

Apart from evaluation of legumes using natural abundance of ^{15}N and ARA, several other nodule performance parameters such as nodule number, nodule mass (Fenta et al., 2011; Pazdernik et al., 1996) or nodule scoring have been used. These parameters have a direct relation with SNF. Complimenting data with ^{15}N natural abundance might provide a better understanding of the performance of legumes. Using these methods in combination with other nodule performance parameters as well as morpho-physiological performance traits could contribute to a more thorough evaluation of the performance of legumes under water deficit.

1.4 Rationale for study

Drought has a significant influence on overall performance of grain legumes as summarized in Figure 1.9, and cause significant yield loss. There is however a possibility to minimize the problem through appropriate research. Identification and measurement of plant performance traits related to drought are fundamental to select superior performing legumes in drought affected areas. Although extensive research has been conducted for searching aboveground physiological and morphological traits for better performance under drought, plant root architectural plasticity and the role of symbiotic nitrogen fixation have not been investigated so far in great detail. These suggests, sustainable grain legume production can be achieved through investigation and application of physiological adaptation mechanism through systematic (root and shoot plant performance) approach (Figure 1.9) and integrating these finding to the existing legume improvement program. Therefore, for understanding both underground (root system) and the aboveground (shoot) basis of plant performance, identification of critical plant morpho-physiological performance traits (markers) for drought tolerance is still urgently needed. This study sought to evaluate the potential of such markers to select the best performing lines under drought, by comparing different common bean and soybean lines.

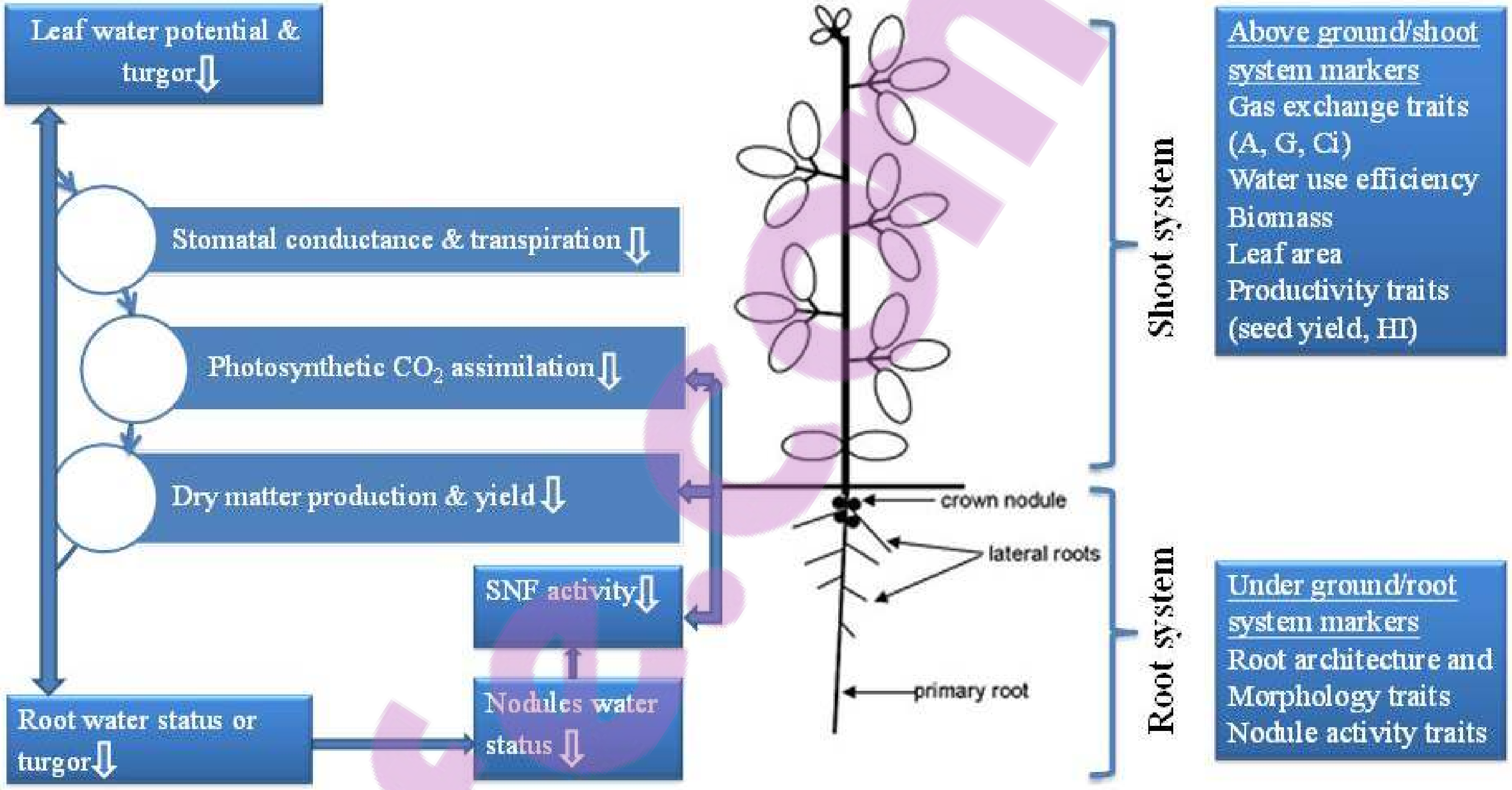


Figure 1.9: Summary schematic diagram of the possible effect of water stress on legumes overall performance and suggested phenotypic markers for legumes performance evaluation. Downward arrows indicate decrease compared to the well-watered state SNF: symbiotic nitrogen fixation, A= CO_2 assimilation, G=Stomatal conductance, Ci= Intra-cellular CO_2 concentration, HI=harvest index.

1.5 Working hypothesis and aim of the study

The overall scientific hypotheses of this study were that:

1) Both common bean and soybean have a similar morpho-physiological phenotypic basis of drought adaptation allowing the application of identical performance markers for selection of drought tolerant cultivars under both controlled and field growth conditions.
2) Root and nodule markers associated with SNF would allow the the best performing common bean and soybean cultivar under drought to be selected.
3) There is a direct relationship between water use efficiency, measured by carbon isotope discrimination, and symbiotic nitrogen fixation, determined by ^{15}N natural abundance,
4) Water use efficiency and symbiotic nitrogen fixation are strongly correlated with seed yield under well-watered and drought conditions .
5) Plant performance under well-watered and drought condition is identical under controlled environmental conditions and field conditions.

The overall aim of this PhD study was to investigate the performance of different bean and soybean cultivars under drought, and to evaluate selected morphological or physiological phenotypic traits (markers) for their potential as markers to select superior cultivars under drought.

The specific objectives of the study were (i) to determine performance of different bean inbred lines and soybean cultivars under water deficit conditions to identify easily measurable plant performance parameters that are associated with drought tolerance under controlled growth condition. (ii) to identify root and nodule markers associated with SNF that will allow the selection of the best performing cultivar under drought (iii) to verify if there is a direct relationship between the carbon isotope discrimination as well as ^{15}N natural abundance with seed yield, root traits and nodule performance under well-watered and drought conditions in common bean lines (iv) to evaluate root and shoot traits under drought stress to determine the physiological basis of differences in growth and seed yield of three soybean cultivars and (v) to evaluate if data obtained by growth of common bean and soybean cultivars in a phytotron are comparable to field data.

CHAPTER TWO

CHARACTERIZATION OF DROUGHT TOLERANCE TRAITS IN NODULATED SOYBEANS

This chapter has been published by:

Fenta, B.A., S.P. Driscoll, K.J. Kunert, and C.H. Foyer (2011)
Characterization of Drought-Tolerance Traits in Nodulated Soya Beans: The Importance of Maintaining Photosynthesis and Shoot Biomass under Drought-Induced Limitations on Nitrogen Metabolism. Journal of Agronomy and Crop Science 198:92-103.

I was responsible for conducting the trial by setting up the experiments and analyzing the data. I was trained by Simon Driscoll/Leeds University to carry out and analyze the infra-red gas analysis study (CIRAS-1, PP Systems Hitchin Herts, UK) and FluorPen fp100 system (Photon Systems Instruments, Brno, The Czech Republic). After I analysed and wrote the draft article, the co-author Prof Christine Foyer helped me in editing the publication.

2.1 Abstract

Drought is the single most important factor limiting soybean (*Glycine max* L. Merr.) yields in the field. The following study was therefore undertaken to identify phenotypic markers for enhanced drought tolerance in nodulated soybeans. Leaf and nodule parameters were compared in three genotypes: Prima 2000, glyphosate-resistant A5409RG and Jackson, which had similar shoot biomass and photosynthesis rates at the third trifoliate leaf stage under water-replete conditions. When water was withheld at the third trifoliate leaf stage, photosynthesis, nodule numbers, nodule biomass and symbiotic nitrogen fixation (SNF) were greatly decreased. Significant cultivar-drought interactions were observed with respect to photosynthesis, which also showed a strong positive correlation with nodule SNF, particularly under drought conditions. Prima leaves had high water use efficiencies and they also maintained high photosynthetic electron transport efficiencies under long term drought. Moreover, Prima had the highest shoot biomass under both water-replete and drought conditions. A-5409RG was the most drought-sensitive genotype showing early closure of stomata and rapid inhibition of photosynthesis in response to drought. In addition to classifying the genotypes in relation to drought tolerance, the results demonstrate that the ability to sustain shoot biomass under nitrogen limitation is an important parameter, which can be easily applied in germplasm screening for drought tolerance in soybean.

2.2 Introduction

Grain legumes, such as peas, beans and soybean are rich in protein, starch, fibre and other essential nutrients, and are valuable in the production of human nutrition and animal feed. The presence of nitrogen-fixing bacteria in specialized organs (nodules) on legume roots means that grain legumes, such as soybean, which has a capacity for nitrogen fixation in the range of 49-450 kg/ha N (Herridge et al., 2008; Wani et al., 1995)can also provide an inexpensive method of natural nitrogen fertilization (Van Heerden et al., 2007). The spatial deployment of roots determines the ability of a plant to secure edaphic resources (De Dorlodot et al., 2007). Root architecture plays an important role in nutrient acquisition with considerable impact on nitrogen use efficiency (NUE) (Garnett et al., 2009). While a correlation between the QTLs for nitrogen uptake and the QTLs for root architecture traits has been suggested (Coque et al., 2008), the involvement of the root traits in NUE is complicated by difficulties encountered when determining the contributions of root systems under field conditions (Garnett et al., 2009). The adverse effects of drought can often be successfully minimized by changing carbon allocation patterns to allow for the formation of a deep root system before the onset of water limitation (Lopes et al., 2011). Thus, it is perhaps not surprising that there is an association between QTLs for root features and plant productivity in terms of yield, water use or nutrient capture (Steele et al., 2007).

The ability to maintain high rates of photosynthesis is an important determinant of the ability of a crop plant to maintain growth during nitrogen limitation (Ding et al., 2005; Vos et al., 2005). Photosynthesis also sustains nodule growth and symbiotic nitrogen fixation (SNF) in legumes

(Voisin et al., 2003). Sucrose produced by photosynthesis in the shoot provides the energy and carbon skeletons required for SNF, ammonia assimilation and the export of amino acids and other nitrogenous compounds. Thus, a significant proportion of the carbon fixed during photosynthesis is allocated to nodule and SNF is a strong sink for photo-assimilates (Silsbury, 1977). The fixed nitrogen originating from SNF in the nodules is supplied to the rest of the plant, via the xylem, as organic N compounds, principally amides and amino acids (Pate et al., 1984). While the nodules of amide-transporting plant species (*Vicia*, *Pisum* and *Lupinus*) contribute relatively more carbon to shoots than do the nodules of ureide-transporting legumes, such as soybean, the transport of assimilates to and from the shoots is dependent on the presence of N_2-fixing nodules and removal of nodules resulted in a large decrease in the transport of the carbon fixed either by photosynthesis or by the nodules via phosphoenol pyruvate carboxylase (Vance et al., 1985).

Grain legume production is severely restricted by drought (Grzesiak et al., 1996; Sincik et al., 2008; Sinclair et al., 2007), which poses a significant threat to food security (Boyer, 1982). About one-third of the world's agricultural land currently suffers from chronically inadequate water availability (Boyer, 1982; Flexas et al., 2006a; Ghannoum, 2009) and this situation is predicted to become progressively worse (Chaves and Oliveira, 2004; Chaves et al., 2003; Jury et al., 2007). The plant hormone methyl jasmonate has been found to enhance the drought tolerance of soybean under conditions of nitrogen fertilization (Anjum et al., 2011). However, very little information is available on the effects of such compounds on nodulated soybeans, which depend on SNF for nitrogen. The legume/*Rhizobium* symbiosis is de-stabilized by drought, which like other stresses causes premature nodule senescence (Matamoros et al., 1999). SNF activity is rapidly inhibited by water deprivation, which causes changes in nodule morphology and metabolism (Fernandez-Luquen˜F. et al., 2008). Drought-induced inhibition of nitrogenase activity is caused by several mechanisms including carbohydrate depletion and feedback regulation by nitrogen accumulation (Serraj et al., 1999). Like chilling stress (Van Heerden et al., 2008), drought can also adversely affect the oxygen diffusion barrier that is crucial to the effective operation of the nodule (Serraj et al., 1999). Oxygen-based limitations on nitrogenase activity occur in soybean nodules even under ambient conditions (Hunt et al., 1989). The permeability of the nodule to oxygen is also influenced by many other factors including water movements into or out of intercellular air spaces (Minchin, 1997; Purcell and Sinclair, 1994; Serraj et al., 1999; Serraj et al., 1995).

Adaptation to drought is a quantitative trait controlled by many different genes. While molecular-genetic approaches are increasingly used to characterise the complex network of drought-related traits in crop species (Manavalan et al., 2009), relatively few applications for DNA marker technologies have so far emerged in practical breeding programs (Lopes et al., 2011). Of the many reasons for the relatively slow uptake of new technologies poor or inadequate phenotyping remains an important constraint (Lopes et al., 2011). Breeding for high crop yields in drought-prone environments is complicated by the year-to-year variability in the amount and temporal distribution of available soil water and the low heritability of drought-resistance traits under these conditions (Lopes et al., 2011). While molecular approaches provide essential candidate gene sequences that allow dissection of QTLs or transgenic approaches to drought tolerance, such approaches require accurate high throughput phenotyping in the field (Araus et al., 2011; Manavalan et al., 2009). The following study was undertaken in order to define physiological markers that could be useful in future breeding programmes for selection of drought-tolerant soybean genotypes and might also be the target for the development of a molecular marker.

In this part of the study it has been investigated if plant traits related to above ground performance, such as stomatal conductance, photosynthesis and biomass is directly related to parameters for nitrogen acquisition in the nodules and can be used for soybean cultivar performance evaluation under drought.

2.3 Materials and methods

2.3.1 Plant material and growth conditions

Seeds of different soybean (*Glycine max* L. Merr.) cultivars Prima 2000, a commercial variety registered in South Africa, A5409RG, a commercial variety used in South Africa that harbours a glyphosate-resistance gene, and Jackson a variety that has nominally been classed as drought-tolerant (Chen et al., 2007; Sall and Sinclair, 1991) obtained from Pannar Seed South Africa (Greytown, South Africa) were inoculated (0.5 g per pot) with a cell powder of the *Bradyrhizobium japonicum* strain WB74-1 (Soygro bio-fertilizer Limited, South Africa). Seedlings were grown in large pots with the volume of 218.2 cm^3 [17.5 cm x 20 cm diameter and 13.1 cm (bottom)] in fine grade vermiculite (Mandoval PC, South Africa), which has a particle size of 0.5-3 mm and a loose bulk density of 100 kg/m^3. This medium consists of thin, flat flanks containing microscopic layers of water (Dupré Minerals Ltd, England). Vermiculite was used specifically in these experiments to provide an N-free medium to facilitate maximal nodule formation under water-replete conditions. Vermiculite has been previously been shown to be the best growth medium for the production of nodules with high SNF activities (Van Heerden et al., 2007) because it avoids problems encountered with soil nutrients that interfere with nodule formation and impair SNF. The plants grown under water-replete conditions were watered twice daily with distilled water and supplied with N-free Hoagland nutrient solution (Hoagland and Arnon, 1950) twice per week.

The experiment was conducted in controlled environment chambers at Forestry and Agricultural biotechnology Institute (FABI), University of Pretoria (-25^0 45′ 20.64″S, 28^0 14′ 8.16″E) during summer season of 2009. The climatic condition of growth condition was, 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 using PAR 2 Meter with SW 11L sensor (S.W & W.S. Burrage, United Kingdom. The indicated PAR is the average of incoming solar radiation measured from 10 am to 3 pm. Furthermore, the supplemental light with a capacity of 350 µmole m^{-2} s^{-2} was supplied with high pressure sodium lamps from 4:00-7:00 pm. The environmental condition in the growth phytotron was monitored regularly to ensure the adequate growth conditions maintained. Eighty soybean plants in total per cultivar were grown to the third trifoliate leaf stage. At this point, half of the plants were maintained under water-replete conditions and half were subjected to drought stress by withholding water and nutrient solution for 18 consecutive days. Pots containing plants under water-replete conditions or subjected to drought randomized throughout each chamber. Further, for all other plant performance traits measurements four individual plants were measured for each water treatment.

2.3.2 Photosynthesis measurements and calculation of instantaneous water use efficiency (IWUE) values

Photosynthetic gas exchange measurements were performed daily on the central leaflet in each case of attached third and fourth trifoliate leaves between 10:00 am to 12:00 am throughout the measurement period using an infra-red gas analysis (CIRAS-1, PP Systems Hitchin Herts, UK) obtained from University of Leeds. Measurements were made at 25^0C and an irradiance of 700 µmol photons $m^{-2}s^{-1}$ and a CO_2 concentration of 350 ± 10 µmol mol^{-1} were used.

Chlorophyll *a* fluorescence measurements were performed using a FluorPen fp100 (Photon Systems Instruments, Brno, and The Czech Republic). For measurement, leaf material was dark-adapted for 20 min by adapting only the part of the leaf which was measured using the leaf clip.

Instantaneous water use efficiency (IWUE) values were calculated as the ratios between CO_2 assimilation rates and stomatal conductance values as described previously (Soares-Cordeiro et al., 2009a).

2.3.3 Leaf water potential

The leaf water potential values were determined from 11:00 -12:00 am during the course of the experimental period using a pressure bomb model 3005 (ICT International, Australia) on the central leaflet that was cut from a fully expanded trifoliate leaf and placed into a chamber with cut ends protruding through the specimen holder. Nitrogen gas was then applied to the leaflet until a drop of sap was observed at the cut end. The pressure required to force a drop of sap from the sample is considered as equivalent to the force with which water is held to plant tissues by forces of adsorption and capillarity (Mario Valenzuela-Vazquez et al., 1997).

2.3.4 Water content of vermiculite

Vermiculite core samples was taken using a cylindrical cork borer (1.4 cm diameter and 11 cm length) every second day. These cores reflect vermiculite water contents to more than half way down the root systems. The fresh mass of the sample was measured immediately using a Model B-502-S Metter Toledo balance (Switzerland). The samples were then placed into a drying oven (Type U 40, Mommert, Germany) at a temperature of 80°C for 24 h. Vermiculite water contents (SWC) were calculated as the difference between the first and second measurements as: SWC (%) = [(fresh mass-dry mass)/dry mass] X 100.

2.3.4 Biomass

Four individual plants (replicates) were harvested and used for distractive biomass measurements. For biomass determination either all vegetative above-ground plant parts (shoot biomass) or all below-ground roots (root biomass) were harvested. Nodule biomass was determined separately after removing the plant roots. Dry biomass of shoots and roots was determined after exposure of plant parts in a drying oven (Type U 40, Mommert, Germany) to a temperature of 80^oC for 48 h.

2.3.5 Nitrogenase activity measurement

Nitrogenase activity was determined using the acetylene reduction assay essentially as described by 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 1ml 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, 1177:180^0C, 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 different level of ethylene.

2.3.6 Statistical analysis

CO_2 assimilation, stomatal conductance, leaf water potential and vermiculate water contents during the duration of the experiment were analysed using Sigma plot 2001, Version 7.0 (1986-2001 SPSS Inc.). The analysis of variance was performed using a JMP® 8.02 statistical package (SAS Institute Inc., Cary, NC, USA). Statistical significance between parameters determined on water-replete and drought treated plants was determined using the LSmeans Student's t-test. The data was further analysed by a bi-variate platform fitting analysis using JMP® 8.02 statistical package (SAS Institute Inc., Cary, NC, USA) software.

2.4 Results

2.4.1 Photosynthesis, stomatal conductance and water use efficiencies

Under water-replete conditions plants of the three cultivars had similar rates of photosynthesis (11.1±0.47 µmol m^{-2} s^{-1} for Prima 2000, 12.1±0.47 for A-5409RG and 10.3±0.42 for Jackson (Figure 2. 1). Photosynthesis (CO_2 assimilation) rates decreased as a result of the imposition of drought in all three cultivars (Figure 2.1). The patterns of drought-induced decreases in photosynthesis varied between the genotypes, for example, a 50 % inhibition of photosynthesis occurred earlier in A-5409RG (Figure 2.1B, about day 4) than in Jackson (at day 9) or Prima (day 11, Figures 2.1A and 2.1C). Prima also had significantly higher rates of photosynthesis ($P< 0.05$) after exposure to long term (at day 18) drought compared to Jackson and A-5409RG (Figure 2.1).

The Fv/Fm ratios were used to determine whether there were any photoinhibitory effects on the electron transport system. However, they were similar in all cultivars under water-replete conditions over 18 d (data not shown) and Fv/Fm ratios remained high in dark-adapted leaves of all three cultivars until day 10 after which this parameter decreased by about 50% in both A-5409RG and Jackson but not in Prima by day 18 ($P<0.05$) (Figure 2.2). These data suggest that damage to the photo-electron transport chain had occurred in A-5409RG and Jackson after prolonged exposure to drought. However, Prima was able to avoid damage to the electron transport system despite inhibition of carbon assimilation (Figure 2.1A).

Under water-replete conditions Prima had the highest stomatal conductance values (Figure 2.3A) and Jackson the lowest (Figure 2.3C) values. Stomatal conductance decreased in all the cultivars when the plants were deprived of water (Figures 2.3). A further analysis of the responses of photosynthetic parameters to drought revealed a significant cultivar-drought interaction with respect to photosynthetic CO_2 assimilation rates, Fv/Fm ratios, and stomatal conductance but not in relation to the intracellular CO_2 concentration within the leaves (Appendix 1).

Jackson had significantly higher ($P < 0.05$) instantaneous water use efficiency (IWUE) values than Prima or A-5409RG under water-replete conditions (Table 2.1). While IWUE values were similar in all genotypes following short term (8 day) drought, Prima had a significantly higher ($P < 0.05$) IWUE than Jackson or A-5409RG after the long term (18 day) drought treatments (Table 2.1). After long term water deprivation, only Prima was able to maintain high IWUE values, whereas in Jackson and A-5409RG IWUE had decreased to levels similar to those observed in the water-replete controls (Table 2.1).

The leaf water potential values were similar in all cultivars under water-replete conditions (Figure 2.4A, control) but decreased progressively in all three cultivars when plants were deprived of water. However, the leaf water potential was significantly higher in Jackson following short term drought than in the other cultivars. The water contents of the vermiculite were similar in all cultivars under water-replete conditions but the values declined progressively after the plants were deprived of water, similar trends being found in all three genotypes (Figure 2.4B).

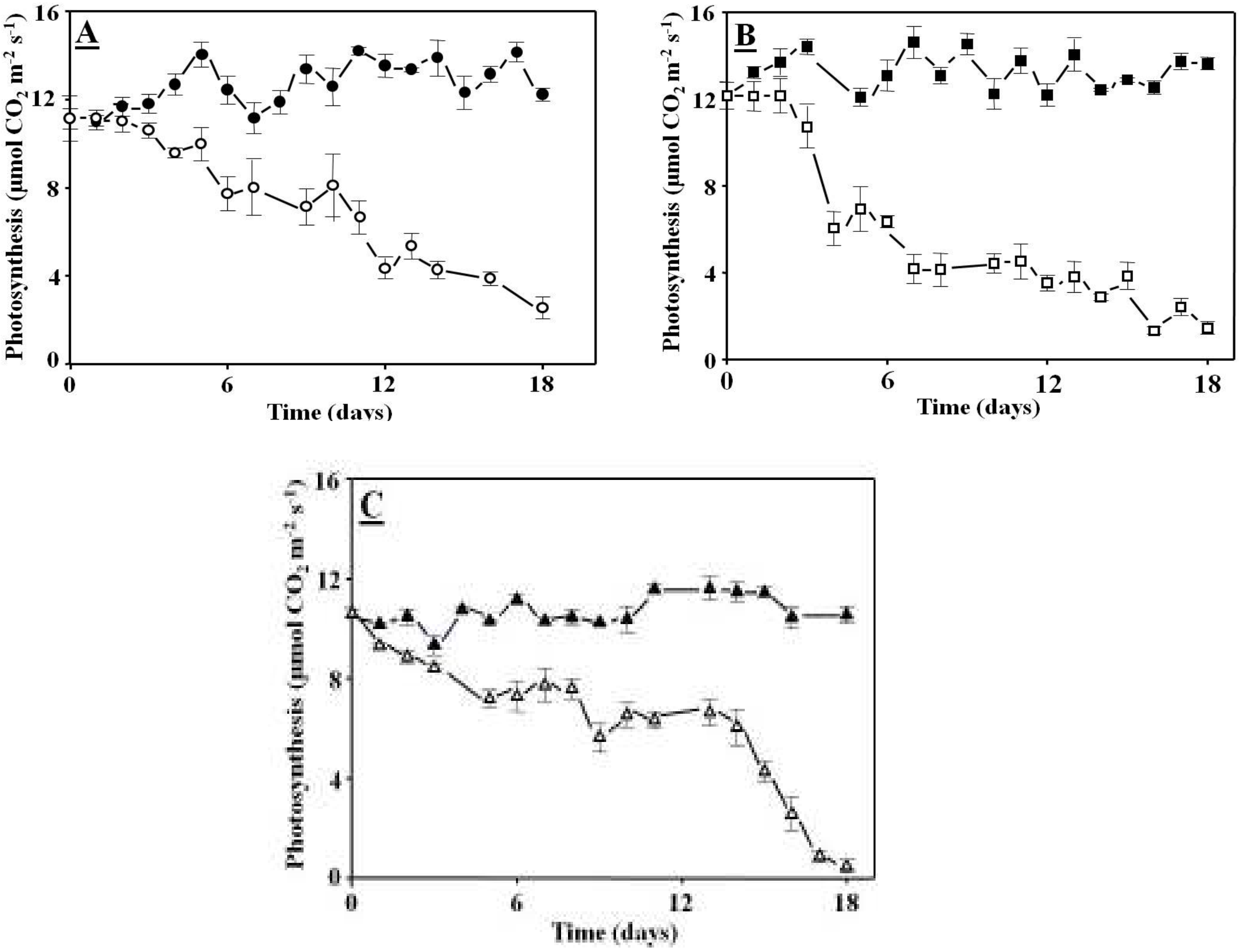


Figure 2.1: Comparison of photosynthetic CO$_2$ assimilation in Prima 2000 (A), A-5409RG (B) and Jackson (C) leaves. Plants were grown under well-watered (closed symbols) and drought (open symbols) conditions for 18 d. Each data point is the mean ± SE from 4 individual plants.

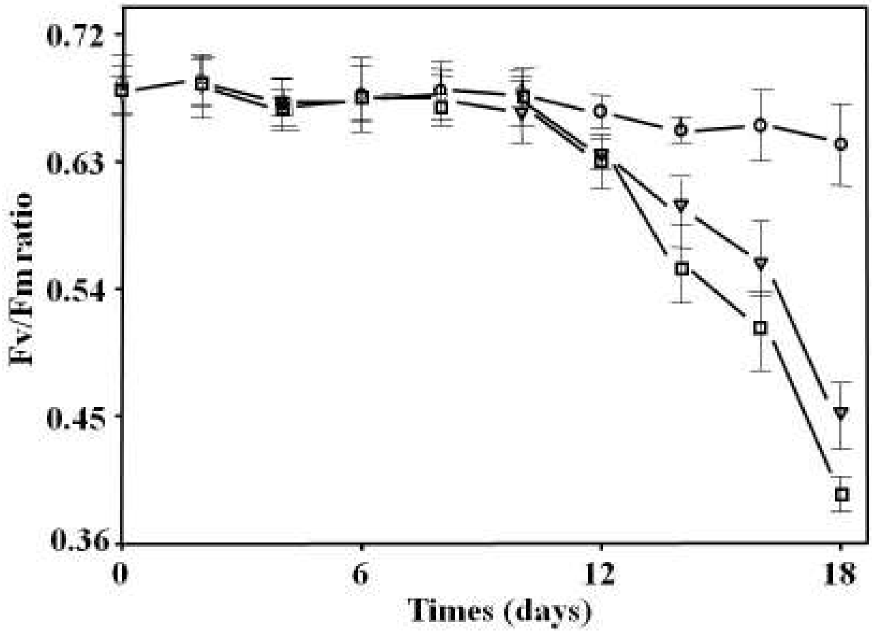


Figure 2.2: Effects of water deprivation on Fv/Fm ratios of leaves of Prima 2000, A-5409RG and Jackson. Plants were grown under drought conditions over 18 d. Circles: Prima 2000, squares A-5409RG, and triangles: Jackson. Each data point is the mean ± SE from 4 individual plants grown under drought conditions. Control values under water-replete conditions were almost identical over 18 d for all three cultivars with a maximal difference between day 0 and day 18 of less than 5%.

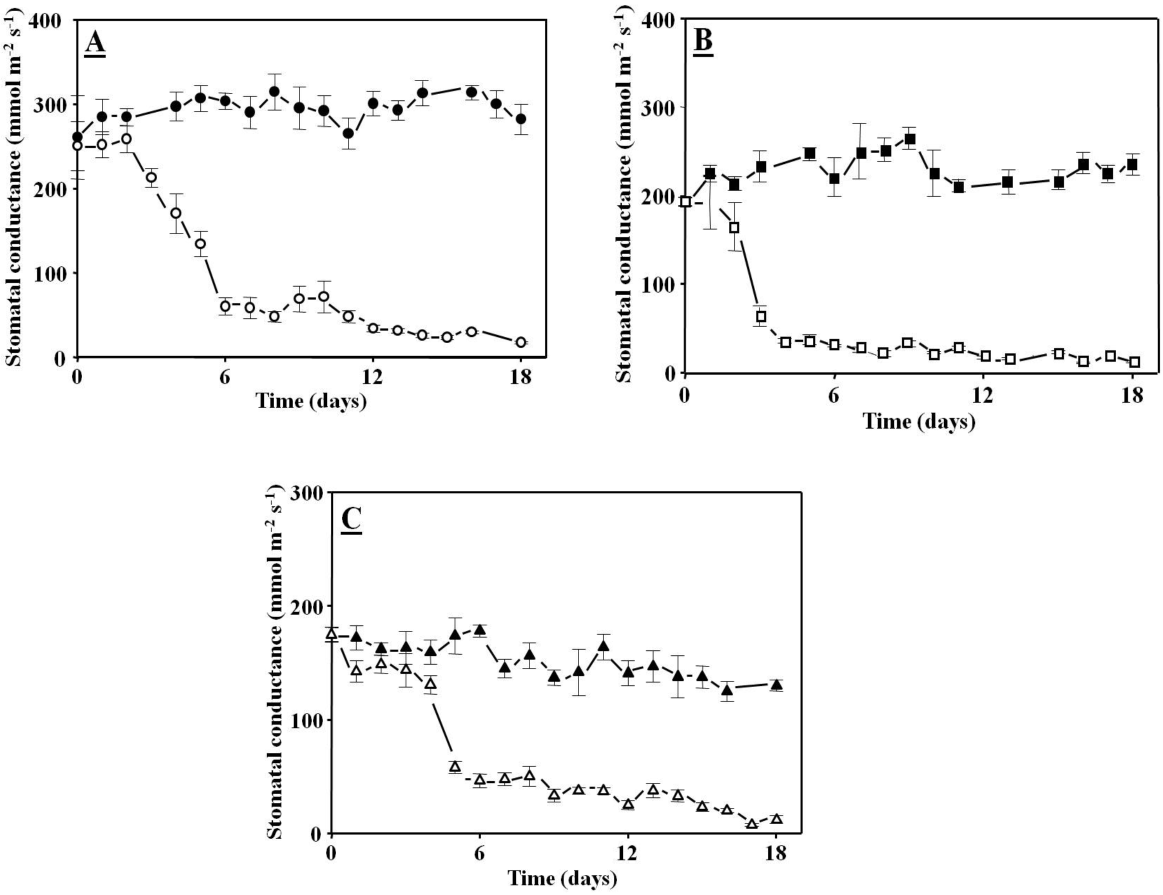


Figure 2.3: Comparison of stomatal conductance values in Prima 2000 (A), A-5409RG (B) and Jackson (C) leaves. Plants were grown under water-replete (closed symbols) and drought conditions (open symbols) for 18 d. Each data point is the mean ± SE from 4 individual plants.

Table 2.1 A comparison of the instantaneous water use efficiency (IWUE) values of leaves of plants of the three soybean cultivars measured under water-replete or drought conditions on day 8 and day 18 of the experiment. Each data point is the mean ±SEM of four independent replicates.

Cultivar	**Instantaneous water use efficiencies (IWUE) (µmol CO_2 per mol H_2O)**			
	8 days		**18 days**	
	Water-replete	**Drought**	**Water-replete**	**Drought**
Prima 2000	49.8±3.1b	186.4±9.2a	73.7±13.7a	156.0±14.0a
A-5409RG	56.5±3.4b	150.7±8.9b	63.2±0.9ab	59.6±13.1b
Jackson	72.4±2.9a	171.1±10.2ab	57.6±1.6b	73.9±4.7b
Significance	**	*	*	**

Different letters in a column denote significant differences (* $P < 0.05$ significant, and ** $P < 0.01$ highly significant).

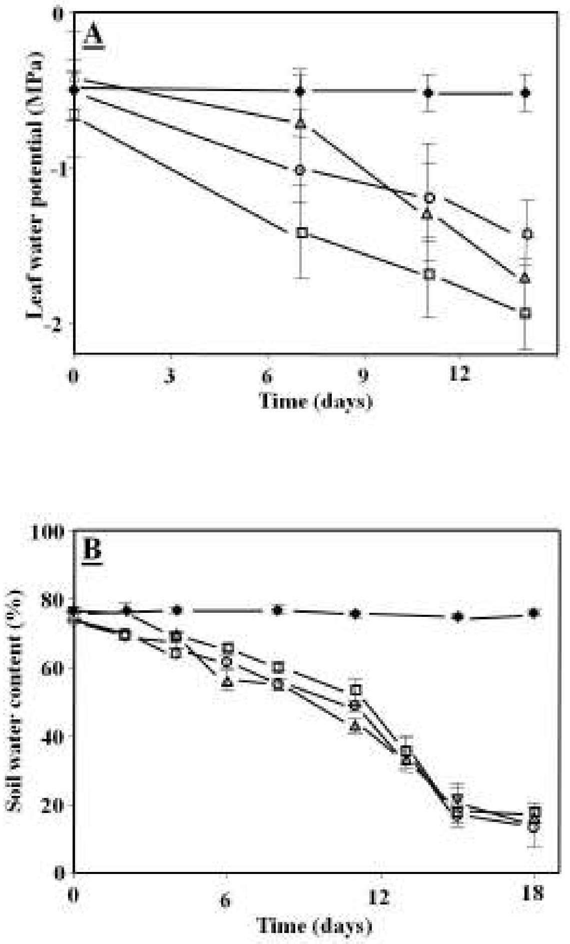


Figure 2.4: Effects of water deprivation on leaf water potential (MPa) values (A) in Prima 2000, A-5409RG and Jackson and vermiculite (Soil) water content as a percentage of the dry soil mass (B). Plants were grown under drought conditions (open symbols) over 18 d. Circles: Prima 2000, squares A-5409RG and triangles: Jackson. Control values (closed diamond) represent the pooled data from all three cultivars using 4 individual plants of each cultivar grown under water-replete conditions. Each data point is the mean ± SE from 4 individual plants grown under drought conditions.

2.4.2 Plant biomass and shoot to root ratio

All three cultivars had similar shoot biomass under water-replete conditions at the start of the experiment (Figure 2.5A) and Jackson had a lower, but not significantly ($P > 0.05$), root biomass value than the other two cultivars under these conditions (Figure 2.5B). Under water-replete conditions, Prima further showed the greatest increase in shoot biomass accumulation over the 18 d experimental period while Jackson showed the smallest increase over this period (Figure 2.5A). A similar trend was observed for root biomass (Figure 2.5B) but there were no significant differences ($P > 0.05$) in the shoot/root ratios of the three cultivars determined either on a fresh or a dry mass basis on day 18 of the experiment under water-replete conditions (Figure 2.5C). Drought treatment led to a significant decrease ($P < 0.05$) in shoot biomass accumulation at 18 d period of the experiment when compared to water-replete treatment for this period (Figure 2.5A). After 18 d of drought, Prima had the greatest shoot and root biomass (Figure 2.5A, B). Values for Prima shoot biomass were approximately 1.7-times higher under-water replete and 2.4-times higher under drought conditions than those of Jackson at day 18 (Figure 2.5A). Root biomass was 1.5-times significantly ($P < 0.05$) higher in Prima than in Jackson but not significantly different ($P > 0.05$) in Prima and Jackson after 18 d of drought in Prima than Jackson after 18 days of well-watered conditions, but not significantly different ($P>0.05$) in Prima and Jackson after 18d of drought with the lowest values obtained in A-5409RG.

The drought treatment resulted in a change in biomass partitioning between roots and shoots with a decrease in the ratio under drought and a great proportion of biomass being partitioned to the roots under drought conditions in all three cultivars (Figure 2.5C). The shoot/root ratios measured on a fresh mass (FW) basis fell from values of 2-3 under water-replete conditions to values of 1-2 after 18 d of drought. The shoot/root ratios measured on a dry mass (DW) basis fell from values of 3-5 under water-replete conditions to values of less than 0.8-1.5 after 18 d of drought (Figure 2.5C). A-5409RG had the highest and Prima the lowest shoot/root ratios expressed either on fresh mass or dry mass basis after 18 d of drought (Figure 2.5C). Furthermore, wilting or leaf rolling was observed in Jackson and A-5409RG but not in Prima under drought stress conditions.

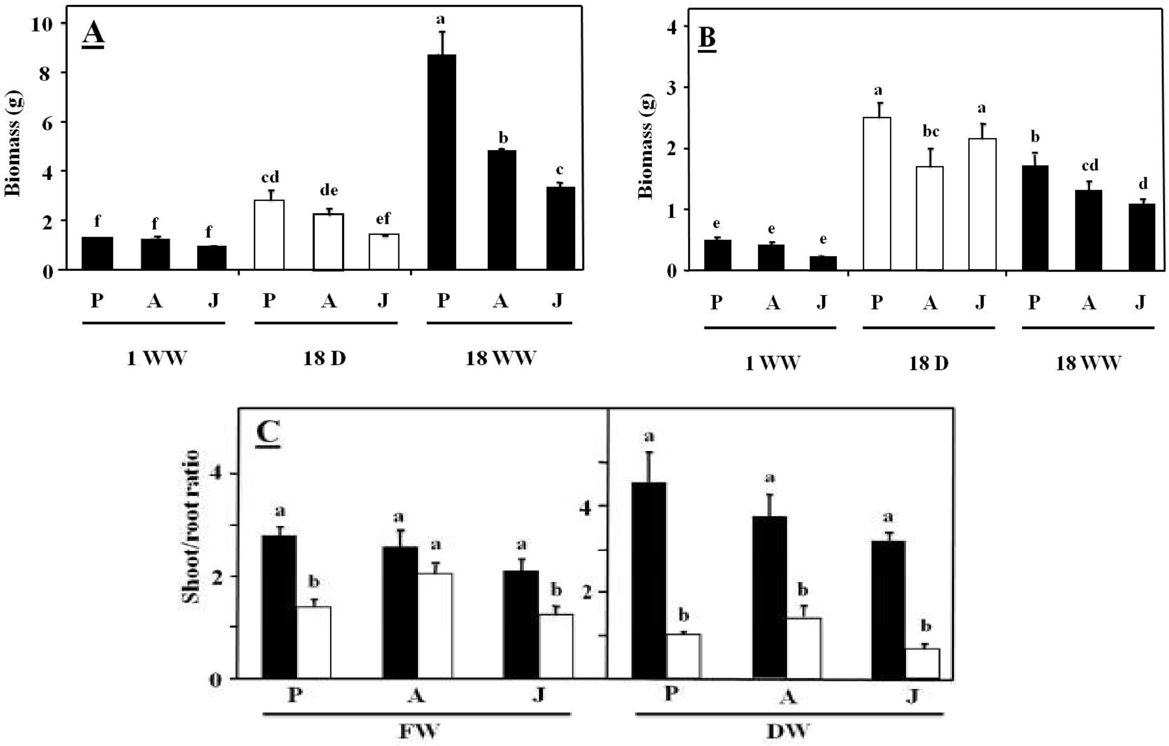


Figure 2.5: Effects of drought on shoot and root biomass and on shoot/root ratio. Shoot biomass (dry mass, A), root biomass (dry mass, B) and shoot/root ratio (fresh mass and dry mass, C) were compared in Prima 2000 (P), A-5409RG (A) and Jackson (J) under water-replete conditions at day 1 (1WW) and day 18 (18WW) and following drought treatment at day 18 (18D). Shoot/root ratios are expressed either on a fresh mass (FW) or dry mass (DW) basis after 18 d exposure to either water-replete (closed columns) or drought conditions (open columns). Each data point is the mean ± SE from 4 individual plants. Different letters denote significant differences.

2.4.3 Nodule parameters and symbiotic nitrogen fixation (SNF)

Prima and A-5409RG had significantly ($P < 0.05$) higher nodule numbers, biomass and SNF under both water-replete and drought conditions than Jackson (Table 2.2). The drought treatment caused a large decrease in nodule numbers in all three cultivars and after 18 d drought treatment, roots had only 11-13% of the nodule numbers when compared to water-replete conditions (Table 2.2). However, after 18 d of drought all three cultivars had similar nodule numbers, biomass and SNF which was not significantly different ($P > 0.05$) between the three cultivars.

Significant positive relationships between nodule SNF and the rate of leaf photosynthesis (Figure 2.6A, $R^2 = 0.67$), stomatal conductance (Figure 2.6B, $R^2 = 0.79$) and leaf intracellular CO_2 concentrations (Figure 2.6C, $R^2 = 0.55$) were observed under water-replete and drought conditions. Significant positive relationships were also observed between SNF and vermiculite water content (Figure 2.7A, $R^2 = 0.68$) and leaf water potential (Figure 2.7B, $R^2 = 0.66$). However, high SNF rates were observed only in a relatively narrow range of high vermiculite water contents and leaf water potential values.

Table 2.2: Comparison of nodule numbers, nodule biomass (fresh mass) and symbiotic nitrogen fixation (SNF) in three soya bean cultivars at 8 and 18 days under either water-replete or drought conditions. Each data point is the mean of four independent replicates.

Cultivar	**Number**		**Biomass (g)**		**SNF (μmol h^{-1}/per plant)**	
	8 d	**18 d**	**8 d**	**18 d**	**8 d**	**18 d**
Well watered						
Prima 2000	56.3±1.1a	136±10.9a	1.5±0.03a	3.1±0.3a	1.9±0.1a	1.9±0.9a
A-5409RG	45.5±1.6b	154±5.7a	0.9±0.1b	2.4±0.2a	1.6±0.35a	1.0±0.03b
Jackson	33.3±3.3c	93.2±5.7b	0.6±0.02c	1.3±0.1b	0.6±0.02b	1.0±0.15b
Significance	**	**	***	*	**	**
Drought						
Prima 2000	33.3±1.8a	15.8±3.7	0.56±0.03a	0.16±0.03	0.85±0.07a	0.06±0.04
A-5409RG	23.5±2.1b	20.7±3.7	0.39±0.02b	0.21±0.03	0.31±0.09b	0.03±0.003
Jackson	26.3±1.1b	10.7±1.0	0.48±0.03a	0.13±0.02	0.50±0.01b	0.02±0.016
Significance	**	*ns*	**	*ns*	**	*ns*

Different letters denotes significant differences (* P<0.05 significant, **P<0.01 highly significant, *ns* non-significant).

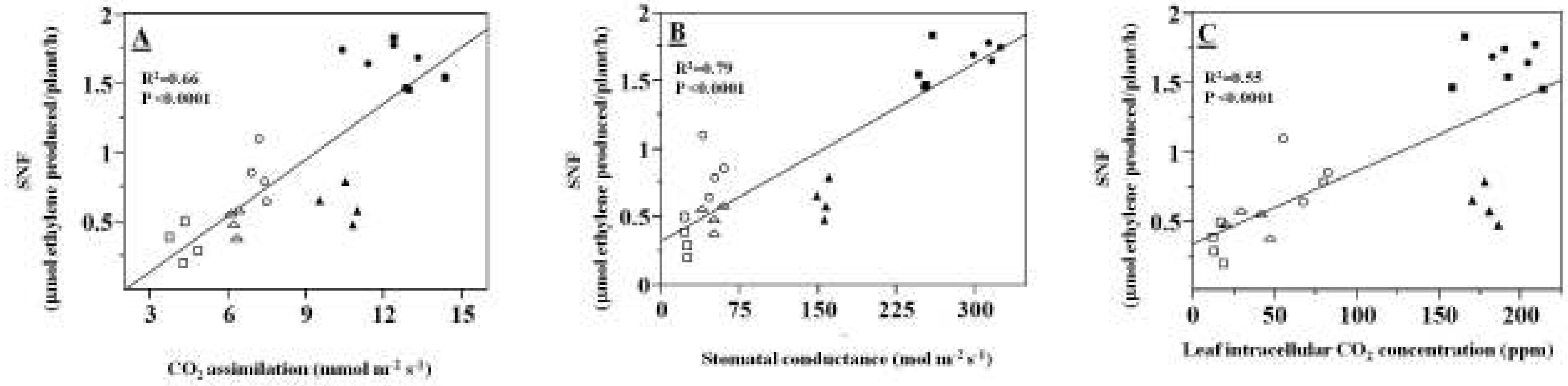


Figure 2.6: Relationships between SNF and CO_2 assimilation (A), stomatal conductance (B) and leaf intracellular CO_2 concentration (C) in Prima 2000 (circles), A-5409RG (squares), and Jackson (triangles) under well-watered (closed symbols) and drought conditions (open symbols). Each data point is the mean from 4 individual plants.

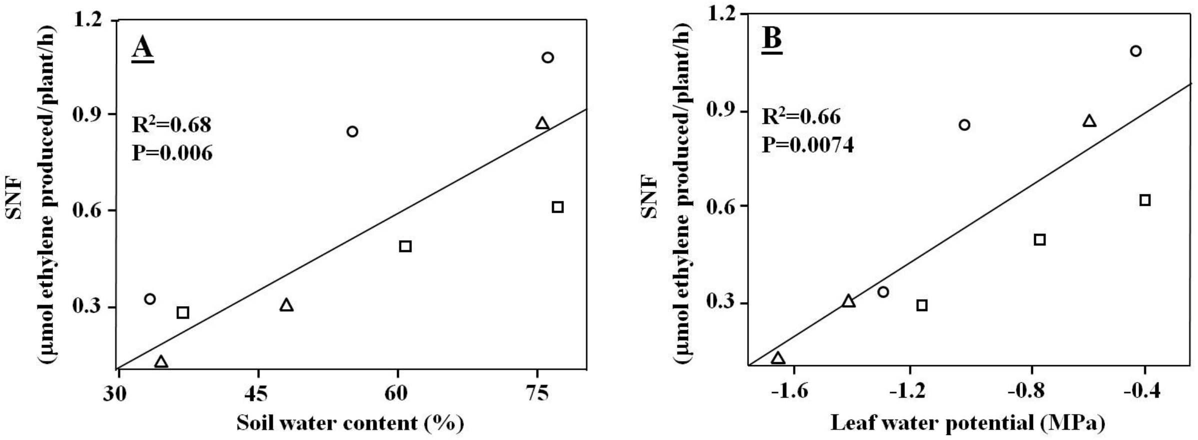


Figure 2.7: Relationships between SNF and soil (vermiculite) water content (A) and leaf water potential (B) in Prima 2000 (circles), A-5409RG (squares), and Jackson (triangles). Data was obtained from plants grown under drought conditions above 30% soil water content or leaf water potentials higher than -1.7. Each data point is the mean from 4 individual plants.

Table 2.3: The R^2 and P-value obtained from the bi-plot regression curve between CO_2 assimilation, stomatal conductance and intracellular CO_2 concentration with soil/vermiculite water content and leaf water potential. The data represents the average of four individual plants measured at the beginning, fist week and day 12 measurements after water stress exposure for both water replete and drought stressed plants of three soybean cultivars.

Trait	**Soil/vermiculite water content**		**Leaf water potential**	
	R^2	**P-value**	**R^2**	**P-value**
CO_2 Assimilation	0.82	<0.0001 **	0.75	<0.0001 **
Stomatal conductance	0.68	0.0005 **	0.64	0.0011 **
Intracellular CO_2 concentration	0.79	<0.0001 **	0.77	<0.0001 **

2.5 Discussion

In legumes, as in other crops, targeted approaches to drought tolerance have often concentrated largely on shoot parameters, particularly those associated with photosynthesis, rather than on root traits, such as nodulation, which are increasingly considered to be important targets for yield improvement in legumes under drought stress. The findings of the present study, in which genetic variation in drought sensitivity was characterized in three nodulated soybean varieties, demonstrate that considerable genetic variation first exists in the ability of soybean to maintain high leaf water use efficiencies and photosynthesis during drought: The data presented here concerning the responses of photosynthesis to drought is further consistent with previous findings (Cornic, 2000; Flexas et al., 2004; Lawlor, 2002b). These authors demonstrated that water stress mainly affects the assimilation of CO_2 predominantly through the decline of stomatal conductance of the leaf which might be also due to co-regulation among these plant processes. Differences in stomatal conductance are considered to exert the greatest effect on the intrinsic water use efficiency (WUE_{intr}) in soybean cultivars (Gilbert et al., 2011). The most marked differences were observed in the ability to regulate leaf water use efficiencies. Jackson had the highest IWUE values under water-replete conditions but only Prima leaves had the capacity to maintain high IWUE values following exposure to long-term (18 days) drought conditions. These data show that the ability to maintain high leaf water use efficiencies will be a useful trait for soybean breeding programs selecting for improved drought tolerance.

The existence of highly significant association of gas exchange traits (CO_2 assimilation, stomatal conductance and intracellular CO_2 concentration) with soil water content and leaf water potential

(Table 2.3) demonstrates as these processes are highly affected by plant water status. Further, these finding ascertains as gas exchange traits are stress sensitive processes and stomatal limitation is mainly as a result of water stress. Thus it is a principal determinant process for decreased photosynthesis of plants under water stress, as it has been also stated before by Chaves (1991) and Baker (1996). These also suggests as a cultivar which maintain better leaf turgor would exhibit enhanced performance for gas exchange traits and maintain plant growth as observed in the cultivar Prima in this experiment.

The stability of photosynthesis under conditions of water deprivation is considered being an important aspect of drought tolerance in soybean (Gilbert et al., 2011). While much controversy remains regarding the relative importance of stomatal and non-stomatal limitations of photosynthesis in drought responses (Chaves and Oliveira, 2004; Flexas et al., 2006b; Lawlor and Cornic, 2002; Warren, 2008), under mild water stress although stomatal limitation of photosynthesis plays a principal role non-stomatal limitations or metabolic impairments such as decreased ATP limits RuBP regeneration and content (Lawlor and Cornic, 2002; Lawlor and Tezara, 2009b) thus deceases carboxylation rate of Rubisco. There is also evidence for increased diffusive resistance within the mesophyll, including the chloroplast conductance for CO_2 (Flexas and Medrano, 2002; Flexas et al., 2004; Signarbieux and Feller, 2011).

Further the cultivar Jackson maintained high rates of photosynthetic CO_2 assimilation over a long period (up to 14 days) of drought exposure. In the first stages of water deprivation, Jackson maintained high stomatal conductance and photosynthesis. These features are consistent with the classification of Jackson as a drought-tolerant genotype (Chen et al., 2007; Sall and Sinclair, 1991). Increase in root mass in Jackson and Prima might be responsible for better maintenance of the leaf water potential, and stomatal conductance and therefore photosynthesis rate.

Photorespiration is considered to increased considerably upon stomatal closure in C_3 plants with a decrease in Ci (Foyer et al., 2009). Photorespiration is a high capacity pathway that acts as an alternative electron sink for the protection of the photochemical apparatus when CO_2 assimilation is limited by low C_i (Foyer et al., 2009). However, with increasing water deficit photorespiration decreases in absolute value (but may increase relative to photosynthesis) and Ci does not decrease to the compensation point (Lawlor, 2002a; Lawlor and Cornic, 2002; Lawlor and Tezara, 2009b). This is because RUBP synthesis is decreased and so limits both photosynthesis and photorespiration. While photosynthetic CO_2 assimilation rates decline following the onset of drought stress, Fv/Fm data show that the photosynthetic light reactions are not impaired and suggest that photo-respiratory carbon flow was able to maintain photosynthetic electron transport rates and protect the photochemical reactions. However, alternative sinks for electrons may also consume electrons and reductant.

In the comparison of the soybean cultivars A-5409RG in this study, Jackson maintained high rates of photosynthetic CO_2 assimilation over a long period (up to 14 days) of drought exposure. In the first stages of water deprivation, Jackson maintained high stomatal conductance and photosynthesis. These features are consistent with the classification of Jackson as a drought-tolerant genotype (Chen et al., 2007; Sall and Sinclair, 1991). Increase in root mass which would be an indicative of root depth and surface area, in varieties Jackson and Prima could be responsible for better maintenance of the leaf water potential as well as stomatal conductance and therefore photosynthesis rate.

Prima leaves had significantly higher Fv/Fm ratios than either A-5409RG or Jackson at the end of the period of water deprivation. This suggest that, although the water potential was maintained for longer for Jackson and Prima, photosystem Prima were less damaged than those two cultivars. This demonstrates that Prima is able to protect the photosynthetic electron transport processes in the leaves more effectively under drought. The maintenance of higher Fv/Fm ratio by Prima suggests that plants of this cultivar have more efficient non-photochemical quenching mechanisms than the others. This protects the photosynthetic machinery from damage. Thus, selecting for more efficient photochemical quenching in drought tolerant plants for example, xanthophyll cycling, which would be important for protecting photosynthetic machinery allowing biomass accumulation under drought conditions and will also allow efficient recovery from drought (Müller et al., 2001).

Symbiotic nitrogen assimilation also shows a strong correlation with photosynthesis: SNF decreases in soybean nodules by stress-induced inhibition of nitrogenase activity (King and Purcell, 2001; King and Purcell, 2005; Van Heerden et al., 2008). Significant relationships were observed between SNF and photosynthetic CO_2 assimilation rates and stomatal conductance values under water-replete and drought conditions in this study. The findings demonstrate that SNF is highly sensitive to water availability in the root environment, and to leaf water potential and photosynthesis. An earlier study reported that the decline in SNF related to soil drying was delayed in Jackson relative to another variety (Serraj et al., 1997). In the present study, Prima had the higher SNF activities and this genotype also accumulated the greatest biomass under both well-replete and drought conditions. Jackson also performed better than A-5409RG under short-term drought conditions but SNF was equally inhibited by long term (day 18 of the experiment) drought in all genotypes.

The value/coefficient R^2 (degree of determination of explained variance) in Figure 2.6 A, B, and C clearly show that the SNF among soybean cultivars is explained by 79, 66, and 55% due to stomatal conductance, CO_2 assimilation and leaf intracellular CO_2 concentration, respectively. The remaining unexplained variance ($1-R^2$) corresponding to that order amounts at 21, 34, and 45%. The magnitude of the unexplained variance are considerable (21, 34, and 45%) showing that the difference in the SNF between cultivars is yet influenced by other additional factors independent to conductance, CO_2 assimilation and leaf intracellular CO_2 concentration. These factors might be a decline in nodule oxygen supply (Denison and Harter, 1995; Minchin, 1997), nodule oxidative stress (damage because of the formation of reactive oxygen species (ROS) (Minchin, 1997) or feedback inhibition of nodule activity due to nitrogen accumulation during water stress (King and Purcell, 2005). These factors also need to be considered in the future during SNF evaluation under drought conditions.

Significant genotypic variation further exists in the control of shoot-root ratios in response to drought. Prima and Jackson were also superior in terms of nodule performance under short-term (first 7 d) drought, in agreement with previous observations for Jackson (Sall and Sinclair, 1991). The better drought tolerance of Jackson has previously been linked to its larger nodule biomass and better supply of photosynthesis to the nodules (King and Purcell, 2001). While cultivar variations in nodules numbers and nodule biomass were observed in the present study, Jackson had the lowest values for these parameters under most conditions. Furthermore, nodule numbers and biomass were in general equally affected by drought in all cultivars. These data show that Jackson performs no better than the other cultivars in terms of sensitivity to long-term drought.

The concept that the adverse effects of drought can be successfully avoided by changing carbon allocation patterns to allow the formation of a deep root system before the onset of a growth-limiting water shortage is widely accepted. This study has shown drought-induced decrease in shoot to root ratios in all three cultivars together with a reduction in whole plant biomass. However, shoot to root ratios under drought were significantly higher in A-5409RG than Jackson or in Prima. This finding shows that there is considerable genotypic variation in the control of shoot to root ratios in soybean in response drought. A recent study revealed considerable flexibility in the control of shoot to root ratios in *Arabidopsis thaliana* in response to another important environmental signal, nitrate (Tschoep, 2010). In the experiment of low nitrate in *Arabidopsis,* Tschoep (2010) found out plants responded to the low N availability by decreasing the shoot growth due the competition of carbon assimilates with the root and thus alters the shoot: root ratio of the plants. In this soya bean experiment for low water availability plants respond for the stress by adjusting the shoot: root growth and the cultivar that respond quickly and efficiently were found to adjust the stress condition and perform better.

The genotypes are ranked as Prima>Jackson> A-5409RG in terms of drought tolerance. Based on the findings reported here, Prima is relatively drought-tolerant because it maintained high leaf water use efficiencies, high Fv/Fm ratios and had the highest biomass under long term drought conditions. This genotype was also able to attain a greater root biomass under drought without severely compromising shoot biomass. On this basis, genotypes like Prima are suitable for cultivation in areas with a longer growing season but suffering drought during the growth period. Shoot biomass is likely to be indicative of seed yield due to the findindings that shoot biomass accumulation in beans strongly correlates with seed yield (Shenkut and Brick, 2003).. Further, in a research conducted in common bean by Shenkut and Brick (2003), the existence of moderate to high heritability of biomass trait and economic seed yield were also ascertained. Therefore, shoot biomass is likely to indicative of seed yields and thus could be an important trait in the selection of soybean germplasm for drought tolerance.

Determinate cultivars with early flowering properties are considered to be well-suited to arid and semi-arid areas. Jackson is considered to be a drought-tolerant cultivar (Serraj et al., 1997) and shows early flowering and seed maturation (data not shown). This genotype is able to maintain a high rate of photosynthetic CO_2 assimilation over a relatively long period of water deprivation, a trait that would be advantageous in conditions where the soil moisture content is adequate early in the season but rapidly declines due to drought. These findings, together with field observations (data not shown) support for classification of Jackson as a drought-escaping cultivar.

Based on all the parameters measured in the present study, A-5409RG was clearly the most drought-sensitive genotype. This glyphosate-resistant genotype had low water use efficiencies, showed early closure of stomata and a rapid inhibition of photosynthetic CO_2 assimilation in response to drought. Furthermore, this cultivar also had the highest shoot to root ratio under drought conditions. These observations suggest that A-5409RG would be better suited to areas that do not experience drought or where crops are grown under irrigation.

The ability to maintain high shoot biomass production under conditions of drought-induced nitrogen deficient might be crucial to sustaining high yields under drought. Prima was able to maintain a higher biomass achieved under drought conditions that result in severe nitrogen deficiency because of impaired SNF. Prima was also able to maintain relatively high rates of photosynthesis under drought conditions and thus maximise carbon gain under conditions of nitrogen deficiency. This trait could contribute to better drought tolerance in soybeans and could be used as a phenotypic marker in breeding programs. While the mechanisms that facilitate rapid growth under limiting nitrogen remain to be elucidated, this trait may be more important in enabling the plants to overcome drought in the field, where nitrogen depletion inevitably accompanies water deprivation.

The characterization of accurate and cost-effective simple phenotypic markers is essential for future legume breeding programs, particularly in Africa. The use of such markers can shorten the selection process for superior plant material with enhanced drought tolerance traits. The present study confirms that in soybean traits related to above ground performance, such as photosynthesis, biomass and stomatal conductance, are directly related to parameters for nitrogen acquisition in the nodules. The ability to maintain vigorous shoot growth under drought-induced nitrogen limitation is an important trait that can be used to select for improved drought tolerance. This trait can be added to the list of potential targets that might be useful in boosting soybean yield gains under optimal as well as drought stress conditions. This list includes improved photosynthetic efficiency, increased sink strength potential and allocation of C and N to developing pods (Ainsworth et al., 2011).

CHAPTER THREE

PERFORMANCE OF COMMON BEAN UNDER WATER DEFICIT IN A CONTROLLED ENVIRONMENT

3.1 Abstract

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	Hardarson G. et al. (1993)
		Fixing more N under drought	Castellanos et al.(1996a); Peña-Cabriales J. and Castellanos (1993)
DOR 364	(BAT 1215 x (RAB 166 x DOR 125)	Drought sensitive	Beebe et al.(1995) CIAT (1996)
		P-sensitive parent	
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 μmol $m^{-2}s^{-1}$ and 400 μmol 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.

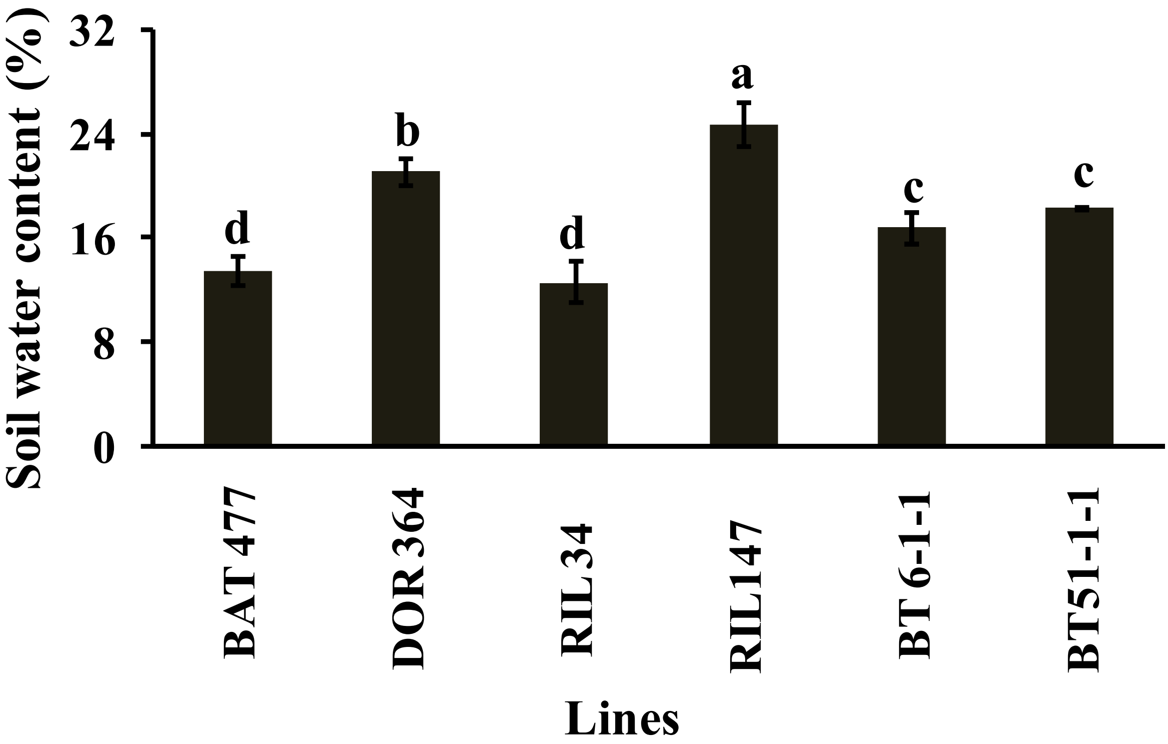


Figure 3.1 Effect of six common bean lines on vermiculite water content after the plants were exposed to water stress for 15 days. Each bar represents the mean ± SE from four individual plants. Different letter on bar denote significant difference ($P < 0.05$). The value indicates the calculated result of the percentage difference of the mass of wet and oven dry vermiculite sample.

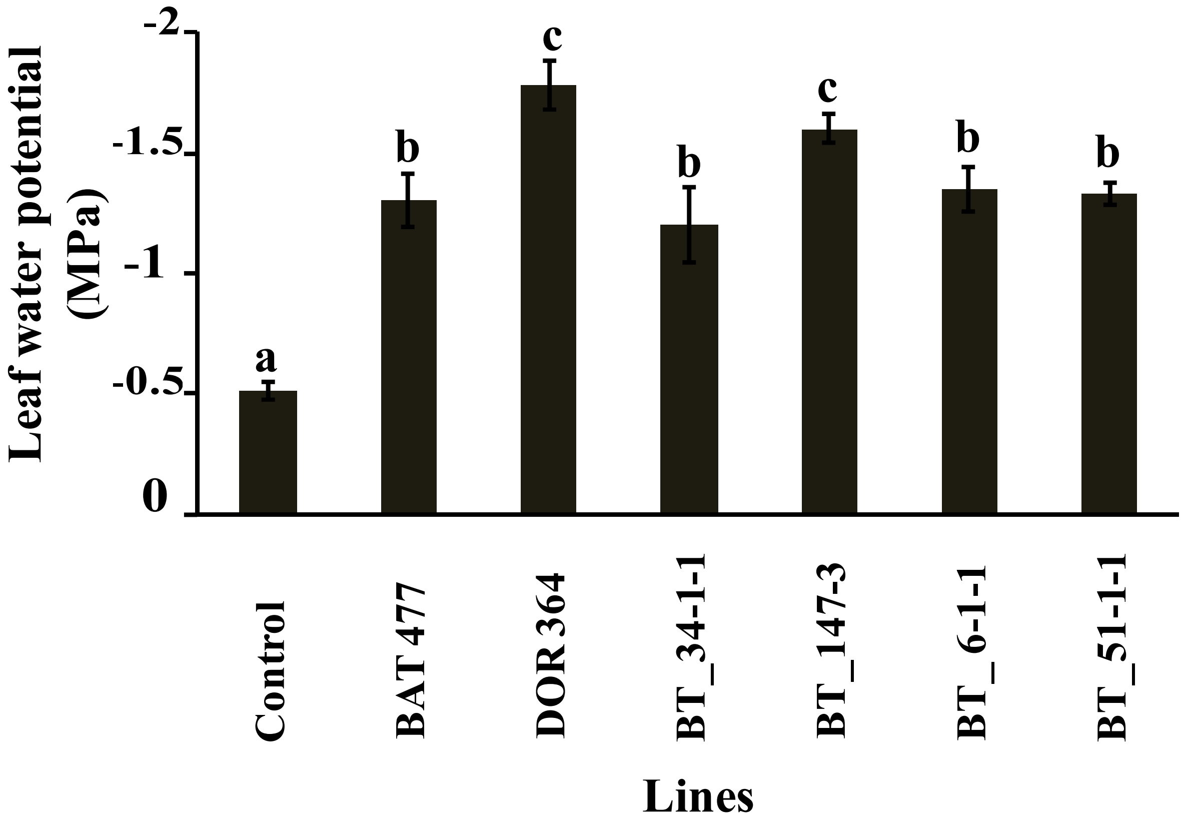


Figure 3.2 Effect of water deprivation on leaf water potential value (MPa) for plants of six bean lines grown under drought. Values represent the mean ± SEM of four individual plants grown under drought for 10 days. Control represents the mean ± SEM of 24 pooled plants (4 plants for each line) grown 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 (µmol $m^{-2}s^{-1}$)**			
	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

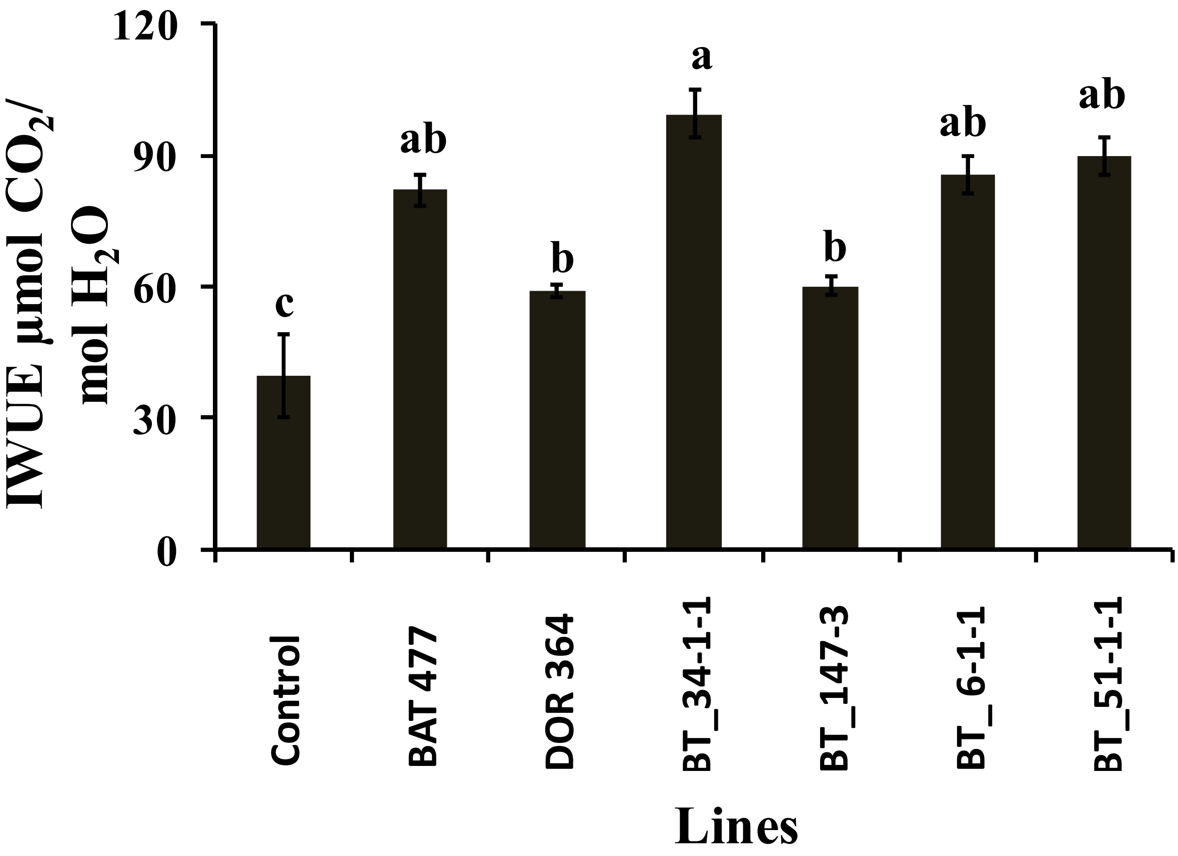


Figure 3.3 Comparison of instantaneous water use efficiency (IWUE) values measured in six common bean lines after 15 days of drought treatment. Data are the means ±SEM of four different plants per lines grown under drought condition. Control represents the mean ±SEM of 24 pooled plants (4 plants for 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) Well-watered

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) Drought

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).

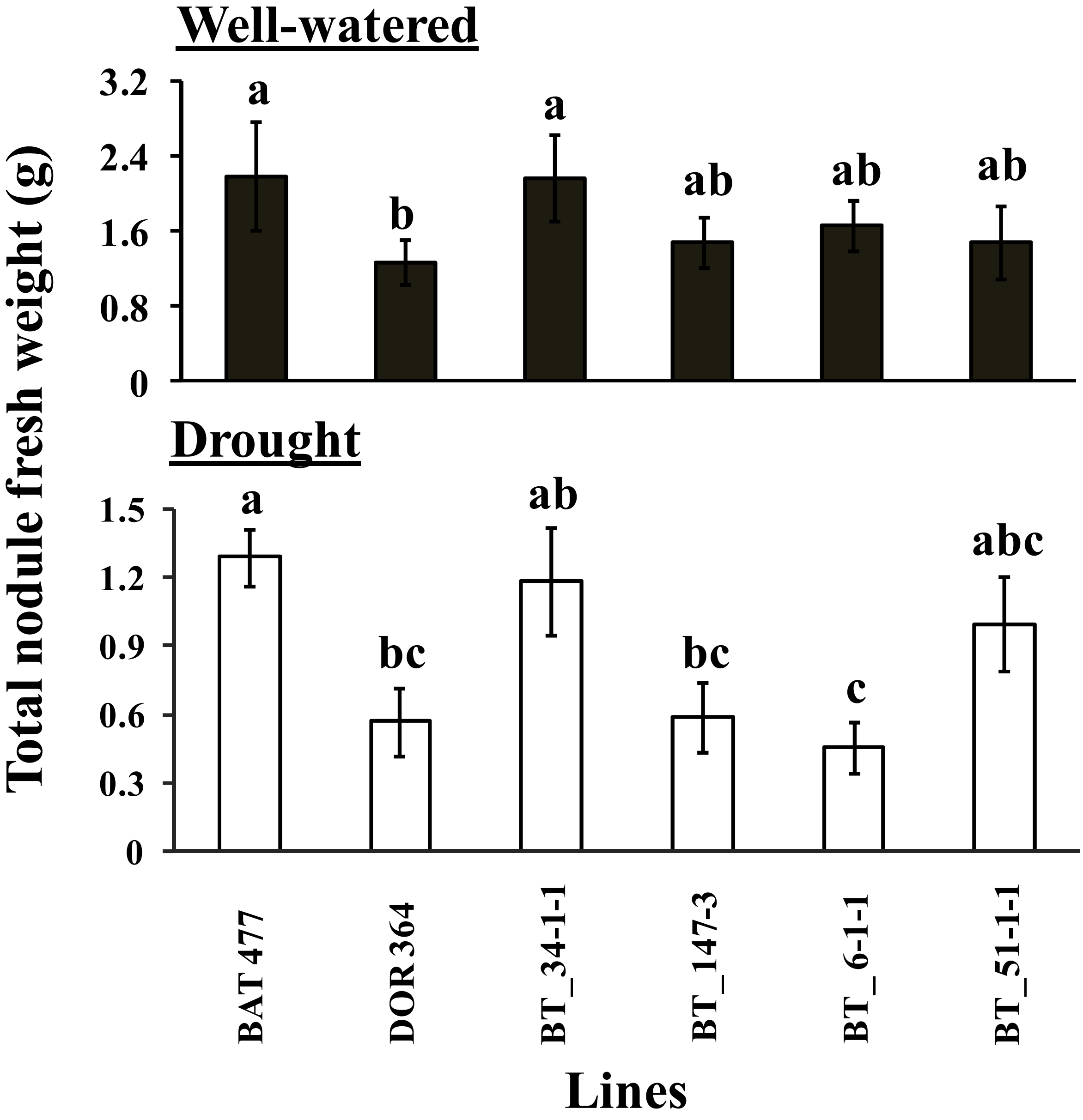


Figure 3.4 Nodules fresh mass of plants of six different bean lines grown either under well-watered or drought conditions. Data represent the mean ± SEM of 4 individual plants. Measurements were carried out 7 and 10 days after exposure of plants to drought and well-watered conditions and individual data obtained from the two time points were pooled. Different letter on bar denote significant difference ($P < 0.05$).

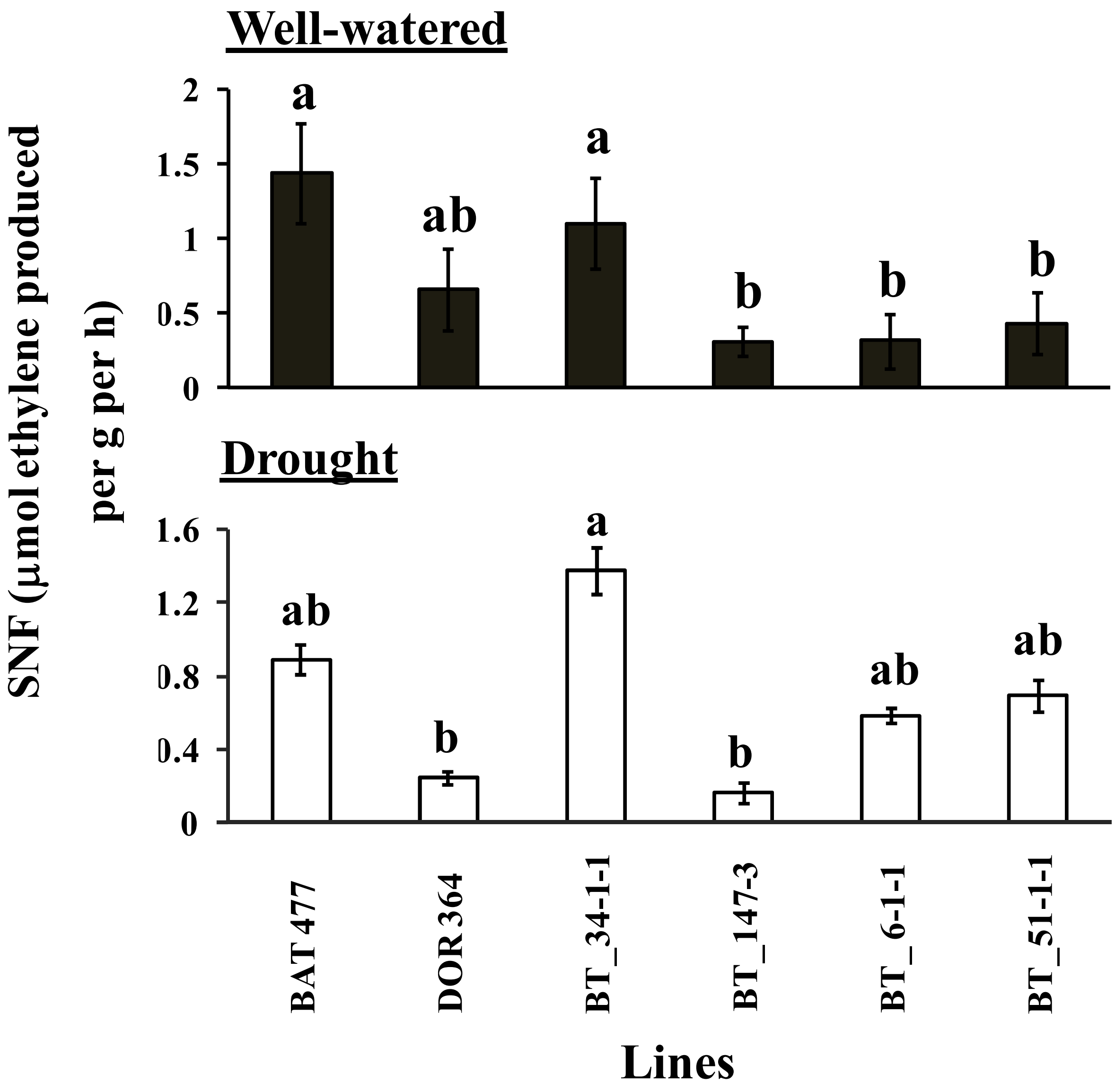


Figure 3.5 Nodule SNF of plants of six different bean lines grown either under well-watered or drought conditions. Bars represent the mean ± SEM of 4 individual plants. Measurements were carried 7 and 10 days after exposure of plants to drought and well-watered conditions and individual data obtained from the two time points were pooled. Different letter within a column denote significant difference ($P < 0.05$).

3.4.5 Nodule performance association with growth and gas exchange

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**
Nodule FW	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**
	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
SNF	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
	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)

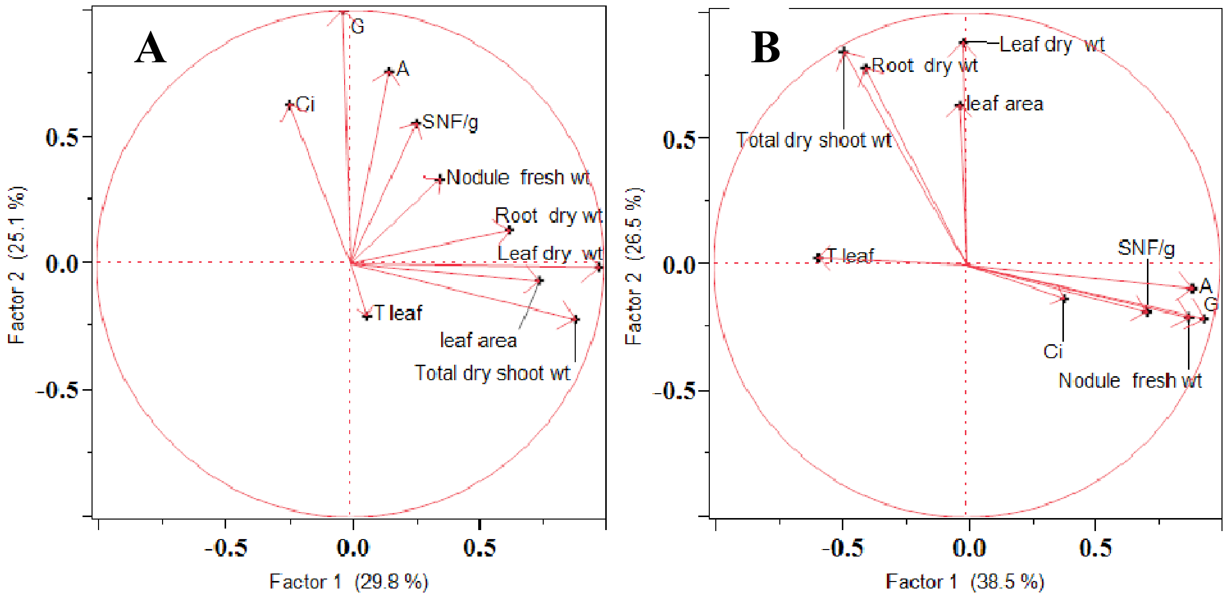


Figure 3.6 Principal component analysis and factor loading plot data of pooled data of the entire measurement period for 10 performance parameters of bean under well-watered (A) and drought (B) conditions.

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 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 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.

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 26-28/13-17^0C 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 7th, 19th and 26th 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 3rd 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).

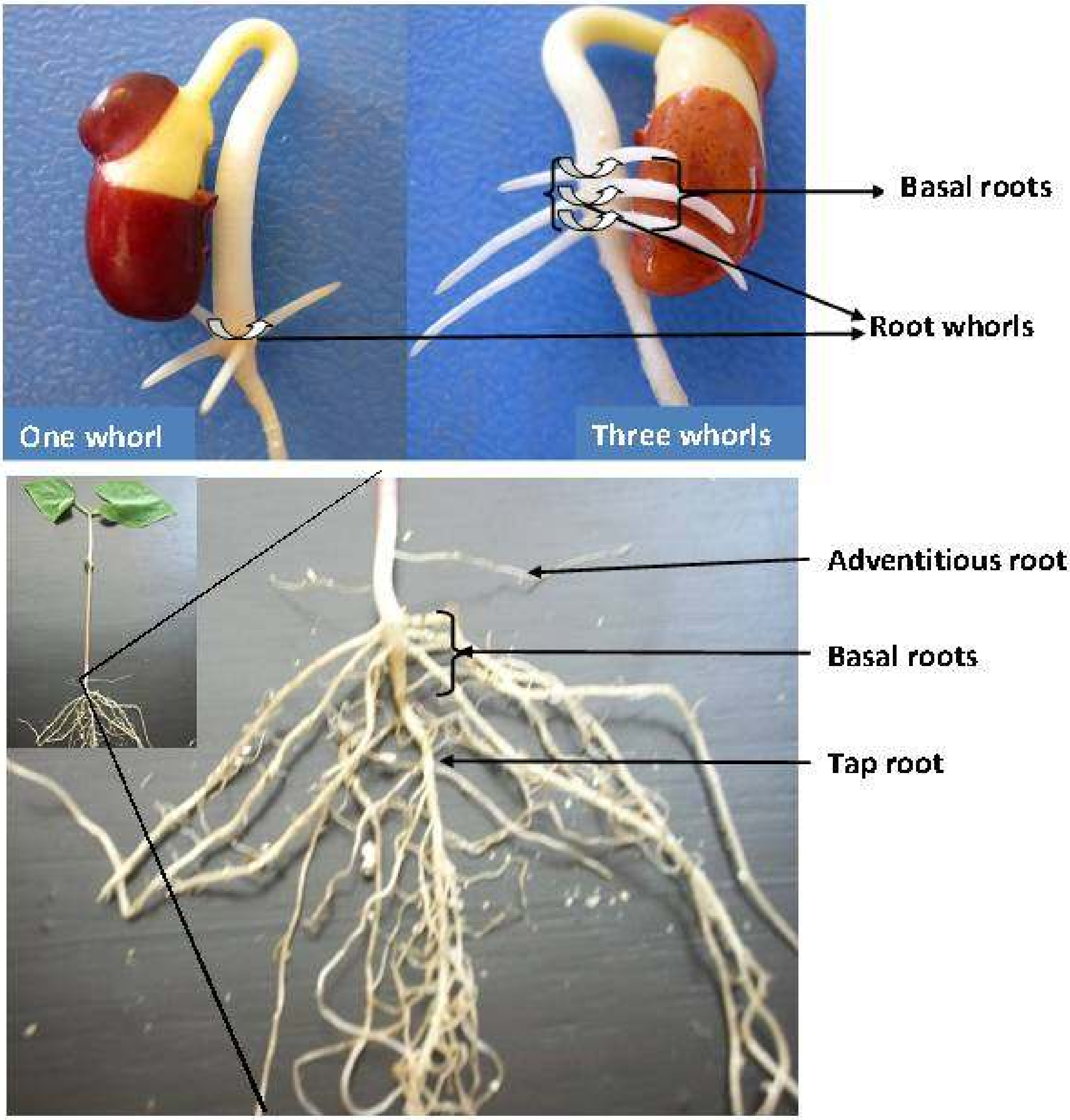


Figure 4.1 Schematic representation of common bean root system architecture with root whorl and main root types.

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.

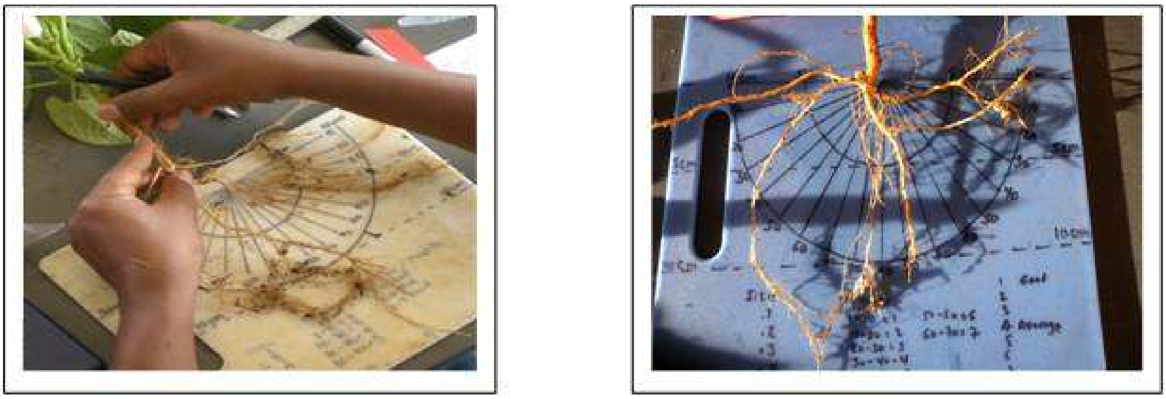


Figure 4.3 Whorl angle measurements by displaying the root on an 180^0 protractor sketched board.

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.

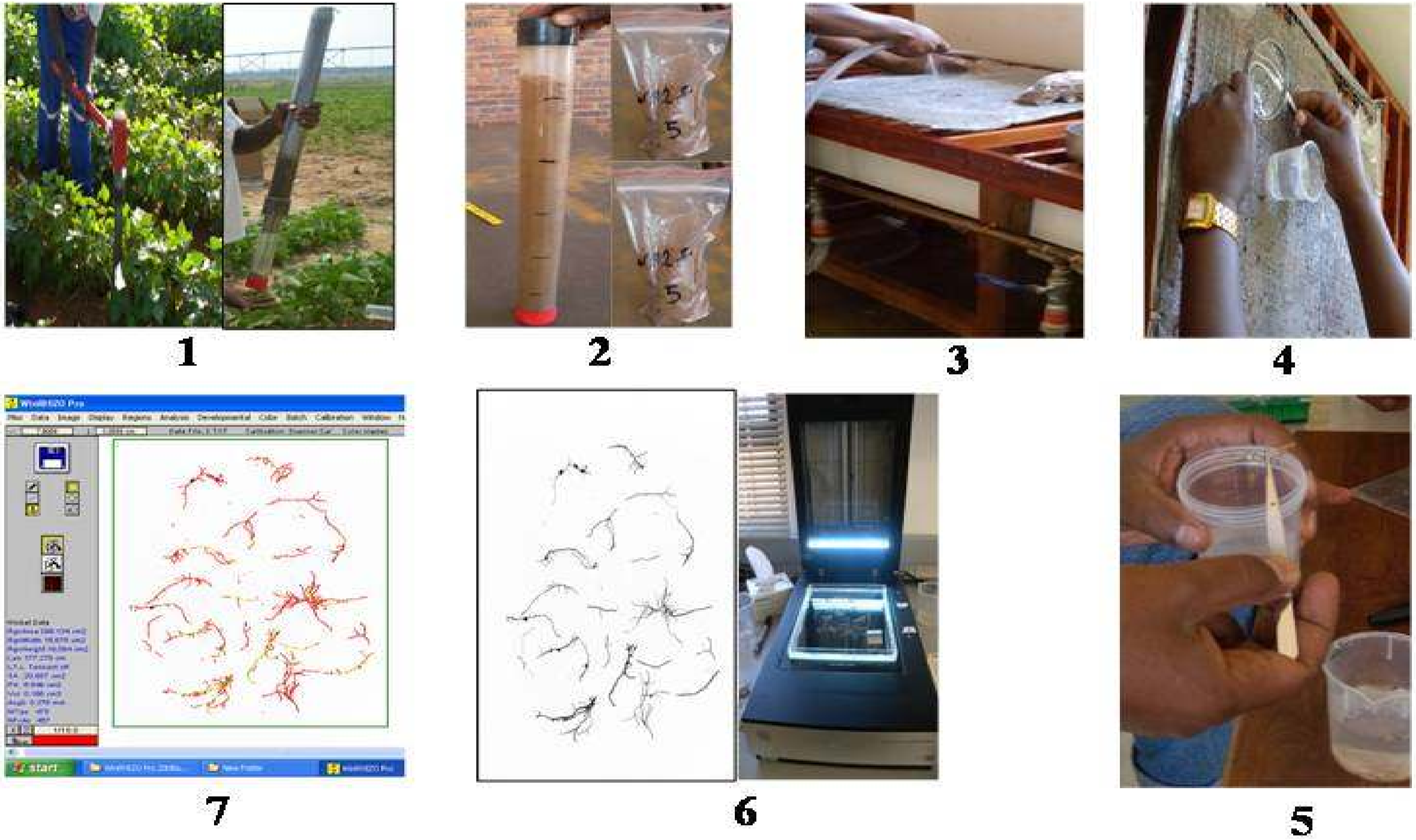


Figure 4.4 Steps involved in root morphology analysis, step 1 (soil coring), step 2 (cutting into pieces), step 3 (washing), step 4 (separating root from foreign materials), step 5 (preserving the root in 25% ethanol), step 6 (scanning root using root scanner Epson Perfection) and step 7 (analyzing the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

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

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.

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

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

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

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^{0}C 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).

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

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).

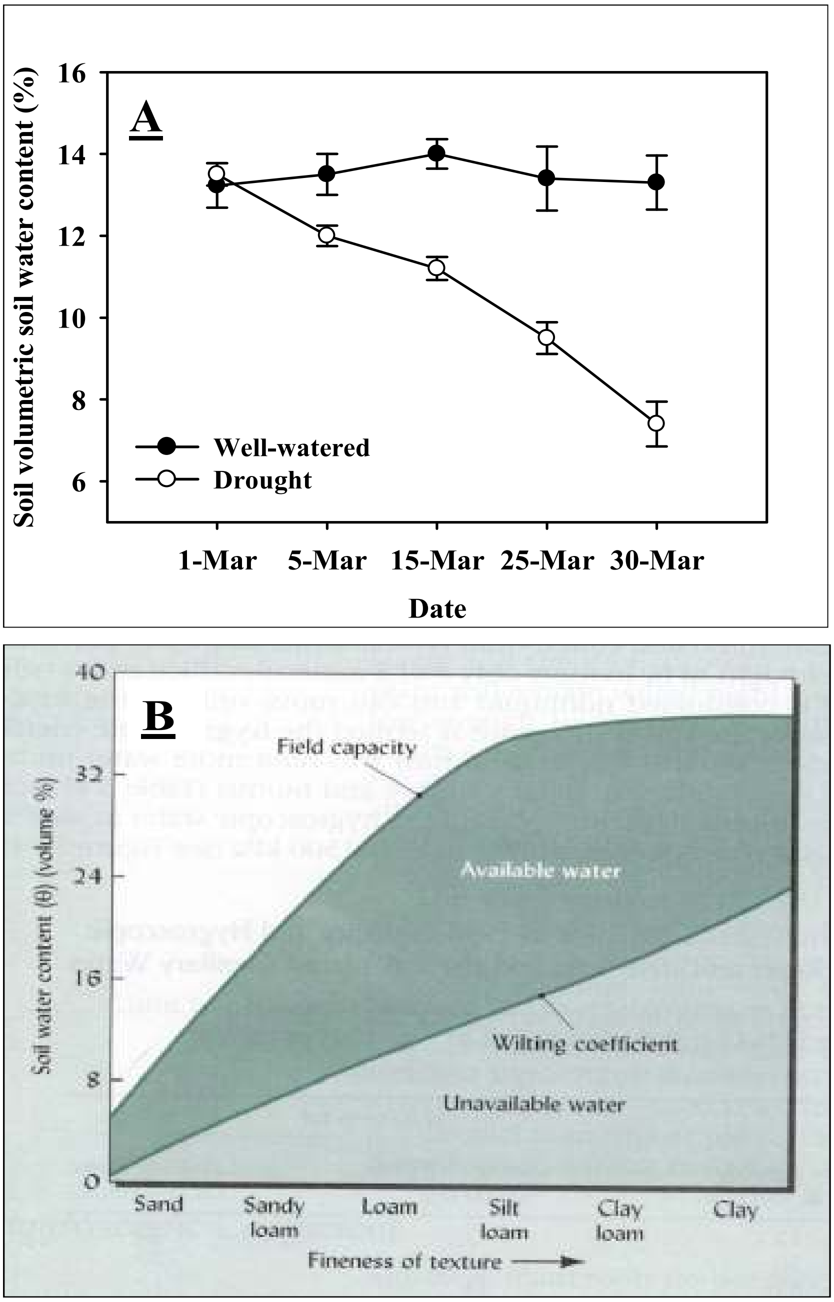


Figure 4.5: (A) Soil volumetric water content values (%) for drought and well-watered blocks on which plants of nine common bean lines were grown. Values represent the mean ± SEM of four soil samples per replication (twelve samples) for each irrigation regime. (B) Soil volumetric water related to soil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

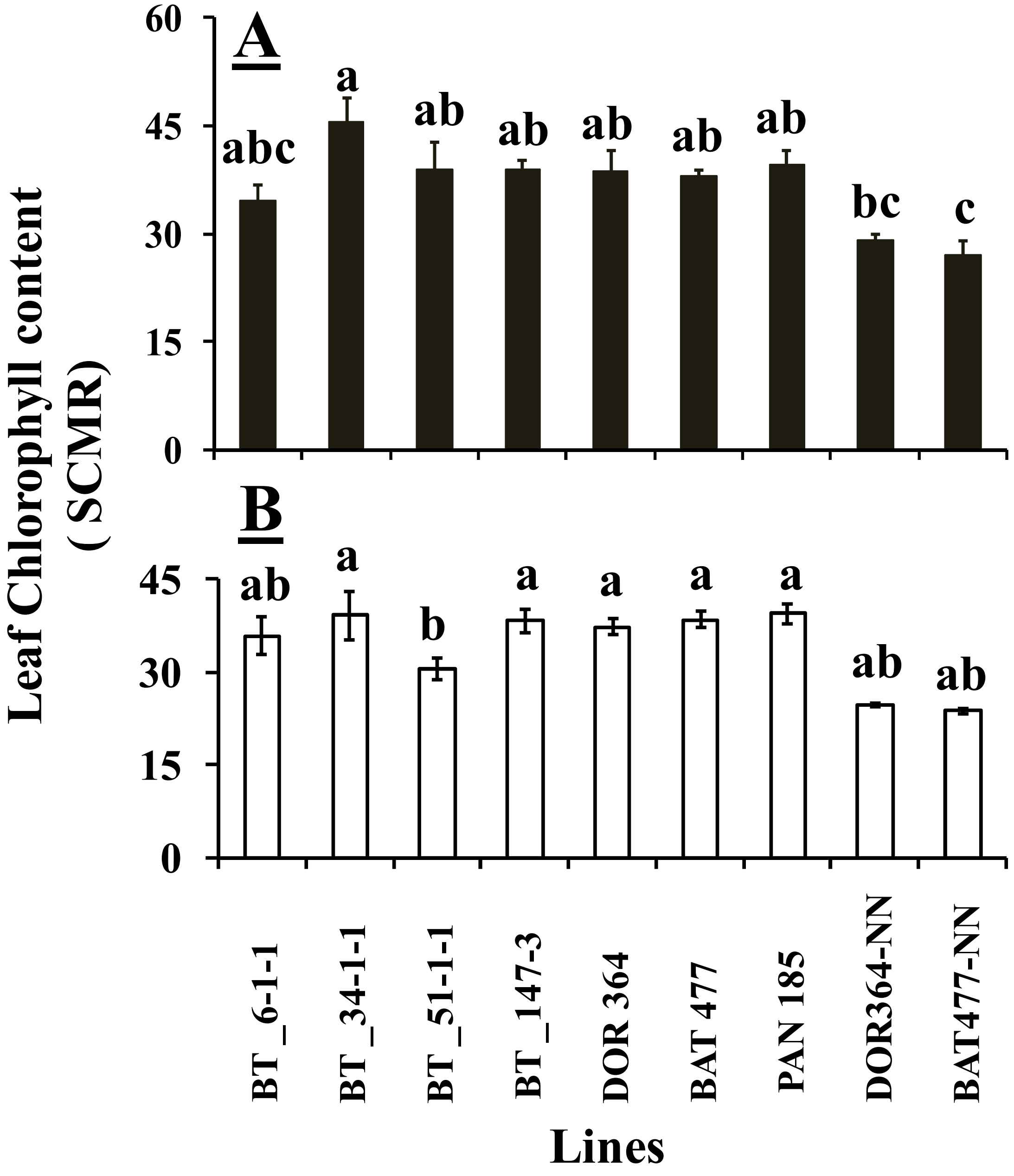


Figure 4.6 Effect of water deprivation on leaf chlorophyll content (SCMR) of nine bean lines measured after three weeks of drought stress at the water-limited treatment. Values represent the mean ± SEM of three plant samples of each variety per plot (nine replicates per treatment) (A) well-watered and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

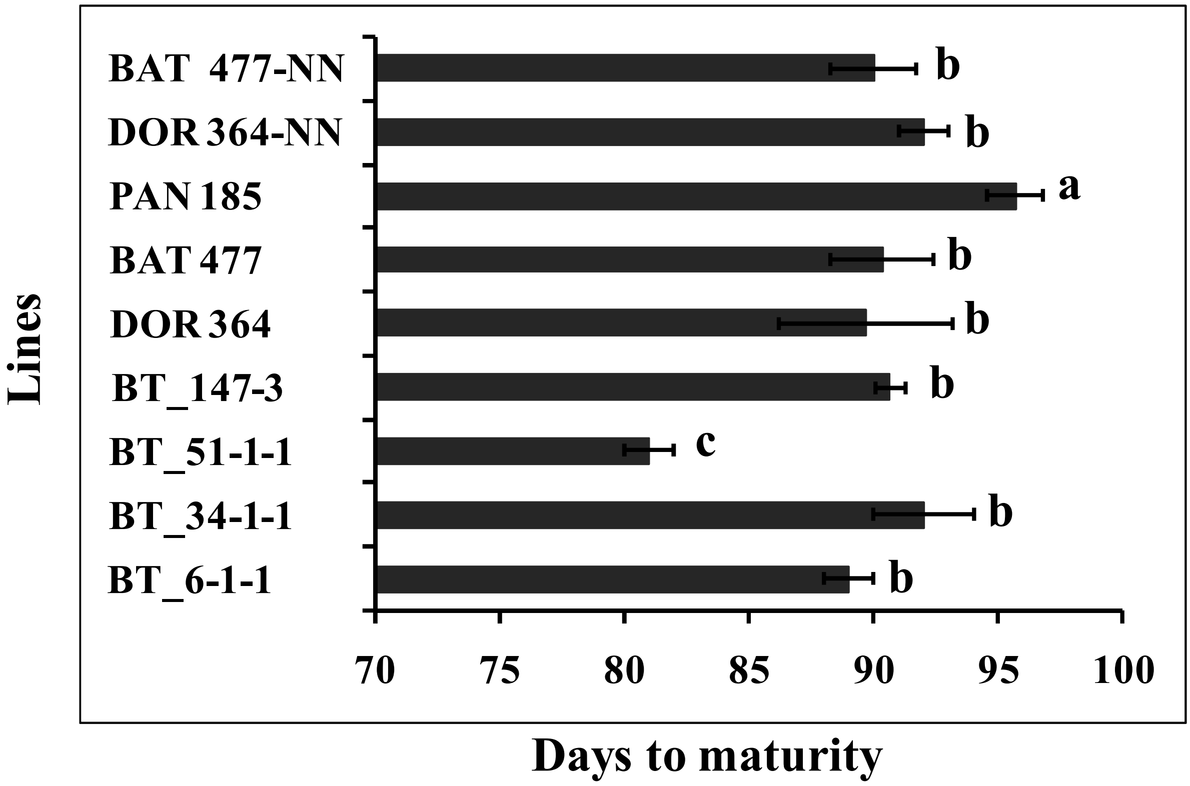


Figure 4.7 Days to maturity of nine bean lines grown under drought conditions. Bars represent the mean ± SEM of each of three plots of each bean lines. Treatment means was determined using the LSmeans Student's t-test and different letter on bar denote significant difference (P<0.05).

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

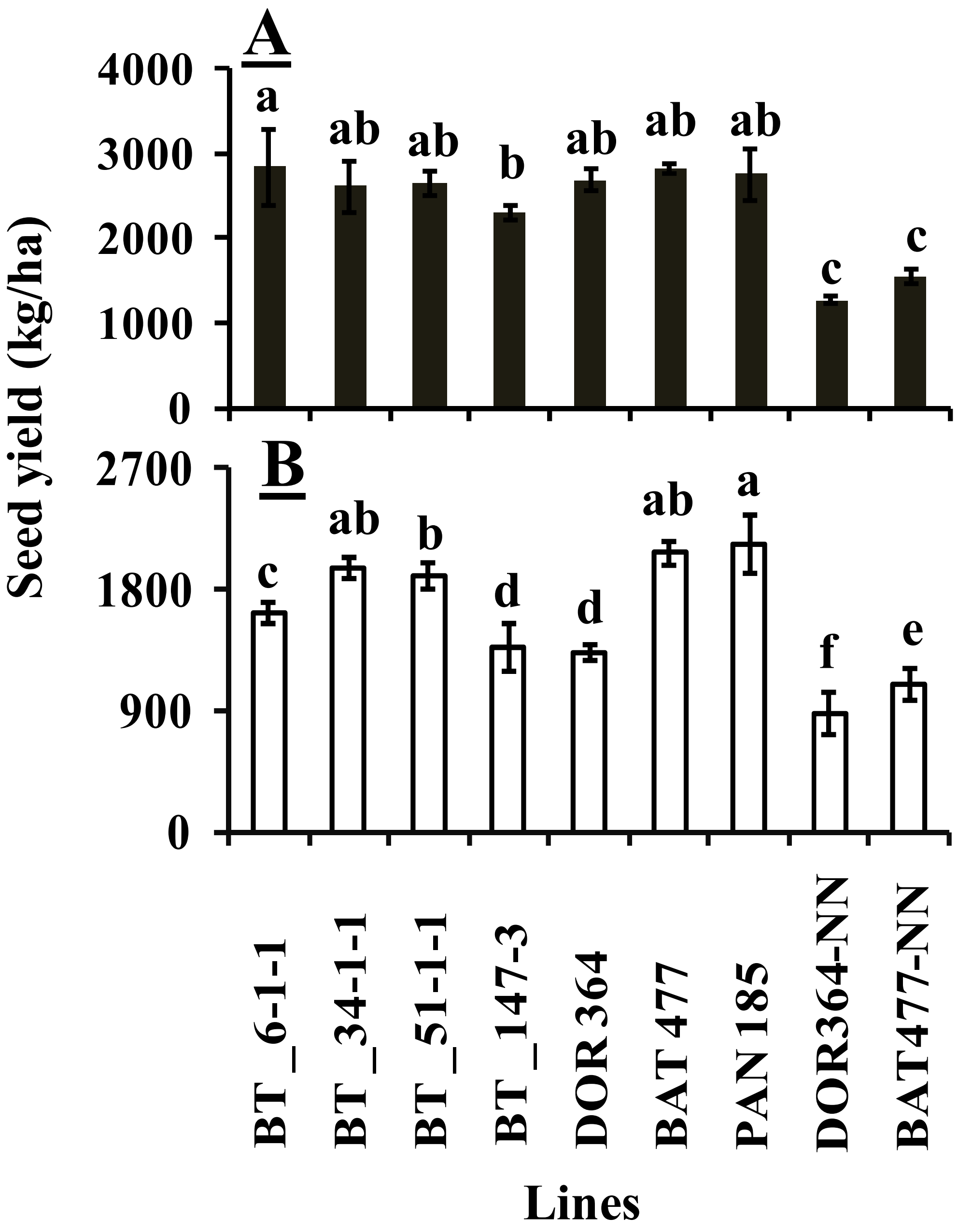


Figure 4.8 Seed yield of nine different common bean lines grown either under well-watered (A, closed bars) or water-limited growth condition (B, open bars). Bars represent the mean ± SEM three replications of two rows of 3 m length for each treatment (2.25 m^2 area) adjusted seed yield at 10% moisture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

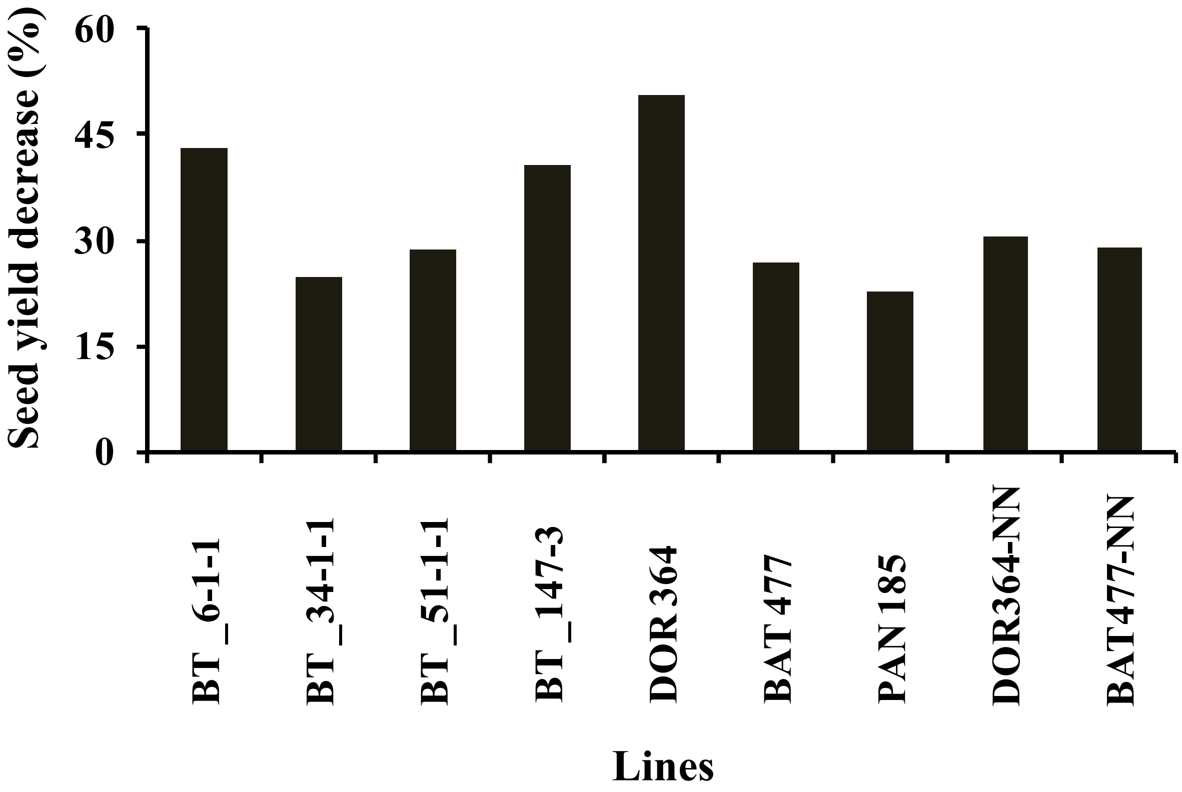


Figure 4.9 Percent decrease of seed yield of nine common bean lines due to water stress. Bars represent the percentage difference of the mean seed yield for three replicates for plants grown under well-watered and water-limited condition in two rows of 3 m length of each treatment. The seed yield was 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 (40-60^0) 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 1st 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.

CHAPTER FIVE

WATER USE EFFICIENCY AND SYMBIOTIC NITROGEN FIXATION OF COMMON BEAN LINES UNDER WELL-WATERED AND DROUGHT CONDITIONS IN THE FIELD

5.1 Abstract

Plant samples were obtained from seven nitrogen-fixing and two non-fixing common bean lines grown in the field. Stable carbon isotope discrimination was determined as a parameter for water use efficiency and natural abundance of ^{15}N together with nodule size measurement for symbiotic nitrogen fixation. Association of these parameters with seed yield, pod harvest index and root morphology and architectural traits was further determined. Performance variation in shoot and seed (CID, C%, δ^{15}N, %N), NDF, plant N and fixed N was found among the tested bean lines. Inbred lines (BT _6-1-1, BAT 477, BT _34-1-1 and BT _51-1-1) and the commercial cultivar (PAN 185) were the best performing. Further, lines with higher carbon isotope discrimination had a higher percentage of nitrogen content in shoots and seeds but lower 15N/14N abundance values under both tested water regimes. The strong relationship found between natural abundance of ^{15}N and carbon isotope discrimination and yield might allow using these two parameters as performance parameters for field-grown beans.

5.2 Introduction

Measuring water use efficiency (WUE) requires determination of the amount of water consumed by the plant which is difficult and time-consuming under field conditions (Martin and Thorstenson, 1988; Rytter, 2005). A stable carbon isotope ratio of 13C/12C in plant tissue has been found to be directly related to WUE which is crucial for enhanced photosynthetic assimilation ultimately determining crop productivity (Rytter, 2005). Carbon isotope discrimination is therefore an indirect way of determining WUE in plants which has been previously applied for WUE germplasm evaluation for different crops (Farquhar and Richards, 1984; Farquhar et al., 1982; Martin and Thorstenson, 1988; Rytter, 2005). Such isotopic variation in C_3 plants is due to discrimination of the diffusion and enzymatic processes in the plant tissue. Farquhar et al., (1982) further found that isotopic discrimination of ^{13}C during CO_2 fixation in C_3 plants is lowest in those plants exhibiting higher WUE. Plants with a lower CID value) assimilate more carbon per unit of water transpired.

In most bean research programs SNF is neglected in the selection of superior performing lines. The ^{15}N natural abundance technique is often used for symbiotic nitrogen fixation (SNF) measurements in the field (Holdensen et al., 2007; Unkovich and Pate, 2000). Generally, There are two types of nitrogen isotopes, ^{14}N and ^{15}N, and SNF is determined by the 15N/14N ratio when the plant ^{15}N concentration is different from the concentration in the surrounding air. The small difference in ^{15}N between the nitrogen-fixing legume and the air (0.3663% atoms ^{15}N) is then used for determining SNF (Holdensen et al., 2007; Shearer and Kohl, 1986; Unkovich and Pate, 2000; Valles-De La Mora et al., 2003). In addition, several other nodule performance parameters including number of nodules, nodule mass or nodule seize can complement measurement of natural abundance of nitrogen (Fenta et al., 2011; Pazdernik et al., 1996).

In this part of the study the question was asked if there is a direct relationship between water use efficiency as measured by carbon isotope discrimination as well as symbiotic nitrogen fixation determined by ^{15}N natural abundance with seed yield, root traits and nodule performance for field grown beans and these relationships would be pertinent for varietal evaluation for water-limited growth condition. Such relationship study in the literature for common beans found to be inadequate. Additionally, it has been also assumed as bean inbred lines performance under control and field condition would be comparable.

5.3 Materials and methods

5.3.1 Plant material

Plant samples were obtained from seven nitrogen-fixing (BT _6-1-1, BT _34-1-1, BT _51-1-1, BT _147-3, DOR 364, BAT 477 and PAN 185) and two non-nodulating (DOR 364-NN and BAT 477-NN) common bean (*Phaseolus vulgaris* L.) lines grown in randomized complete block design (Appendix 3) at the Ukulima Root Biology Center (URBC), operated by Natural Conservation Thrust, Limpopo Province, South Africa.

5.3.2 Parameters measured

5.3.2.1 Nodule size

Root nodule size was determined by placing the multiple root nodules on a board with a sketch of the diameter of nodules.

5.3.2.2 Carbon isotope discrimination / ^{15}N natural abundance

Three replicate plant samples from each plot (individual bean line) harvested after one month drought exposure from the well-watered and drought treatment blocks and used for root phenotyping and dry mass determination from above ground parts (both leaf and stem) were ground to fine powder using a grinder (A 11 basic Analytical Mill, IKA® Works, Inc, Germany). The three samples were mixed and used for carbon isotope discrimination and ^{15}N natural abundance determination.

Isotopic analysis was done at Cape Town University (Department Archaeology). For that, samples were weighed into tin cups to an accuracy of 1 µg on a Sartorius micro balance. The cups were then squashed to enclose the sample. The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). Gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). The in-house standards used were MG-Merck Gel, proteinaceous gel produced by Merck, and dried lentils (purchased from Pick & Pay). All the in-house standards were calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen was expressed in terms of its value relative to atmospheric nitrogen, while carbon was expressed in terms of its value relative to Pee-Dee Belemnite. The following procedures were used to determine these isotopes.

Stable carbon isotope discrimination was determined using the following equation:

$$\delta 13C ‰ = \frac{\mathbf{R}_{sample} - \mathbf{R}_{sandard}}{\mathbf{R}_{standard}} * 1000$$

With R_{sample} and $R_{standard}$ being the abundance ratios 13C /12C ($\delta^{13}C$) of the sample and the standard, Pee Dee Belemnite (PED) (Farquhar et al., 1982; Mostajeran and Rengel, 2007; Rytter, 2005).

Carbon isotope discrimination was computed from $\delta^{13}C$ of each plant sample, assuming the atmospheric $\delta^{13}C$ (δair) was -8‰ using the following (Farquhar et al., 1989) formula and used by different authors (Kondo et al., 2004; Merah et al., 2001)

$$\mathrm{CID}(‰) = \frac{(\delta \mathrm{air} - \delta \mathrm{plant})}{(1 + \delta \mathrm{plant})} * 1000$$

Natural abundance of $\delta^{15}N$ was calculated using the following formula previously reported (Shearer and Kohl, 1986; Unkovich et al., 1994; Valles-De La Mora et al., 2003):

$$\delta 15\mathrm{N}(‰) = \frac{\%\mathrm{atom\ 15N(sample)} - \%\mathrm{atom\ 15Nair}(0.36637)}{\%\mathrm{atome15N\ air}\ (0.36637)} * 1000$$

To calculate the percentage of the nitrogen fixed by the legumes from the atmosphere the following formula was used (Bergersen and Turner, 1983; Shearer and Kohl, 1986):

$$\%\mathrm{Ndfa} = \frac{\delta 15\mathrm{N(refernce\ plant)} - \delta 15\mathrm{N(Nfixing\ legume)}}{\delta 15\mathrm{N(refernce\ plant)} - \mathrm{B}}$$

%Ndfa represents nitrogen derived from the atmosphere, reference plant represents the non-fixing plant used in the experiment and B represents the value obtained from the legume that grows in the medium where atmospheric N_2 is the only source. The B value was obtained using the same four bean lines and rhizobium strain used in this field trial, which was replicated four times(16 samples) and completely dependent on atmospheric N_2 fixation for growth grown under controlled condition in N-free medium and with N-free nutrient solution. The plants samples for analysis for $\delta^{15}N$ were also done at the same stage of the filed grown bean which was at flowering. The value obtained was (-4.10882)

Plant biomass at flowering was measured using six plants per line. Calculations were done for two rows with 3 m length to determine the plant N for harvestable area.

Plant N= (Plant DM)* (%N)/100 (Peoples et al., 2009)

N_2 fixed = (fixing plant N)-(control/non-fixing) (Peoples et al., 2009)

5.3.3 Statistical analysis

Experimental data were analyzed using the JMP® 9.0 statistical package (SAS Institute Inc., Cary, NC, USA). Analysis of variance was carried out to determine the significance level and treatment comparisons via the LSmeans student's t-test. Multivariate Pearson's correlation analysis was used for determining the relationship (correlation) between measured traits.

5.4 Results

5.4.1 Stable carbon isotope discrimination

Stable carbon isotope discrimination analysis was conducted for plant shoots after one month of drought and for seeds after harvest. When analysis of variance was carried out for shoot and seed variables of CID, C% and C: N ratios, but not shoot C%, were significantly different ($P<0.05$) both under well-watered and drought conditions (Appendix 7), however, two way ANOVA (water treatment X lines) for these traits were not significantly different (data not shown).

When a treatment comparison was made for shoot and seed CID as well as seed C% under well-watered conditions, the nitrogen-fixing lines BT_6-1-1, BT_51-1-1 and BT_34-1-1 performed better for shoot CID with significantly lower (higher WUE) ($P<0.05$) CID values than BT_147-3 and DOR 364, while BT_51-1-1 the best performing line (Table 5.1). Under drought, the nitrogen-fixing lines BT_34-1-1, BAT 477, DOR 364 and PAN 185 performed better for shoot CID with significantly higher WUE (lower CID) ($P<0.05$) values than all other tested lines and with PAN 185 the best performing line (Table 5.1).

When seed CID was compared (Table 5.1) under well-watered conditions BT_6-1-1, BT_34-1-1, BT_147-3 and BAT 477 were best performing. Furthermore, under drought BT_6-1-1, BT_34-1-1, BT_51-1-1 and BAT 477 performed better than all other lines. Further, seed C% analysis revealed that under drought BT_6-1-1, BT_51-1-1, BT_147-3, DOR 364 and PAN 185 performed better than all other lines (Table 5.1).

Table 5.2 shows correlation coefficients to determine any association of the productivity traits (PHI and seed yield) with carbon isotope discrimination and C% in shoots and seeds. A significant ($P<0.05$) negative relation was found under drought conditions for both seed and shoot CID with seed yield as well as PHI. However, under well-watered condition significant association of CID with seed yield was found only for seed CID value. Moreover, C% was significantly positively associated with seed yield at well-watered condition only.

Table 5.1 Performance of nine bean lines for carbon isotopes discrimination of shoot and seed samples. Result is the mean of three replicates (each replicate from a composite sample of three plant samples) for each bean lines for each water regime.

Lines	**Shoot** CID		**Seed** CID		**Seed C%**	
	Well-watered	**Drought**	**Well-watered**	**Drought**	**Well-watered**	**Drought**
N-fixing						
BT _6-1-1	21.369±0.15c	20.94±0.23bc	19.330±0.37cd	21.369±0.15c	41.885±ab	41.683±ab
BT _34-1-1	21.564±0.24bc	20.626±0.61c	18.326±0.63d	21.564±0.24bc	42.254±a	42.320±a
BT _51-1-1	21.375±0.27c	20.914±0.28bc	20.006±0.44bc	21.375±0.27c	42.006±ab	41.854±ab
BT _147-3	22.442±0.33a	21.498±0.90a	19.327±0.54cd	22.442±0.33a	41.944±ab	41.577±bc
DOR 364	22.350±0.26a	20.913±0.53bc	20.198±0.76abc	22.350±0.26a	41.547±b	41.706±ab
BAT 477	21.885±0.24abc	20.703±0.13c	18.839±0.73cd	21.885±0.24abc	41.927±ab	41.289±bc
PAN 185	21.859±0.5abc	20.738±0.27c	20.276±0.61abc	21.859±0.5abc	41.627±ab	41.625±abc
Non-fixing						
DOR 364-NN	22.293±0.04ab	21.337±0.01ab	21.47±0.14ab	22.293±0.04ab	40.275±c	40.945±c
BAT 477-NN	22.224±0.29ab	21.677±0.73a	21.688±0.07a	22.224±0.29ab	40.886±c	41.150±bc
Significance	0.0372*	0.0032**	0.006**	0.0372*	<.0001**	0.0327*

Significance level was determined using ANOVA (** *P<0.001*, **P<0.05*, and ^{ns}*P>0.05*) and difference between treatment means were determined using the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different.

Table 5.2 The relationship of CID and C% (shoot and seed) with productivity traits (seed yield and pod harvest index) for plants grown under well-watered and drought conditions. Data for CID and C% were obtained from three replicates (each replicate from a composite sample of three plant samples) of each bean line and water regime. Pod harvest index (PHI) and seed yield were determined from three replicates of either one row (PHI) or two rows (seed yield) per plot.

Trait	**Productivity traits**			
	PHI		**Seed yield**	
	Well- watered	**Drought**	**Well- watered**	**Drought**
Shoot CID	-0.323	-0.419	-0.325	-0.525
	ns	*	ns	*
Shoot C%	0.236	-0.079	0.235	0.167
	ns	ns	ns	ns
Seed CID	-0.398	-0.544	-0.525	-0.425
	ns	*	*	*
Seed C%	0.534	0.275	0.680	0.183
	ns	ns	*	ns

r = Pearson's correlation coefficient

* indicates the correlation is significant *($P<0.05$)* and ns= indicates the correlation was insignificant (*$P>0.05$*)

5.4.2 Nitrogen fixation

When nodule size of different bean lines was measured under well-watered and drought conditions, largest nodules size under well-watered conditions was found for the nitrogen-fixing lines PAN 185, BAT 477, BT_34-1-1 and BT_6-1-1 with BT_6-1-1 having the largest and DOR 346 the smallest nodule size (Table 5.3). Under drought, nodule size was reduced for all bean lines when compared to nodule size under well-watered conditions with the lowest reduction for PAN 185 and the highest for BT_6-1-1 and BT_147-3 (Table 5.3)

Two way analysis of variance (water treatment X lines), for the nodule performance traits was not significantly different (data not shown). Therefore, one way ANOVA and treatment comparison has been use to assess the performance of bean lines. Accordingly, analysis of variance for shoot and seed $\delta^{15}N$, %N and nitrogen fixation efficiency parameters (Ndfa % N shoot, Plant N and Fixed N /2.25 m^2) revealed significant differences under both well-watered and drought conditions, except for Ndfa %N shoot under well-watered conditions (Appendix 8). There was no significant difference ($P>0.05$) among N-fixing lines for $\delta^{15}N$ in shoots under well-watered conditions (Table 5.4A). Further, the highest (low SNF) (1.73) and the lowest (high SNF) (-1.45) shoot $\delta^{15}N$ were found for the non-N fixing bean lines (BAT 477-NN) and BT_34-1-1, respectively. However, under drought, N-fixing lines significantly differed ($P<0.05$) in shoot $\delta^{15}N$ with lines BAT 477, BT_6-1-1, BT_34-1-1 and BT_51-1-1 having the lowest and the non-N fixing line DOR 364-NN the highest $\delta^{15}N$ value (Table 5.4A). For seed $\delta^{15}N$ under well-watered conditions, lines BT_34-1-1 and BT_51-1-1 had the lowest and the non-N fixing lines BAT 477-NN and DOR 364-NN the highest seed $\delta^{15}N$ and these differences were highly significant ($P<0.05$). Under drought, the non-N fixing lines BAT 477-NN and DOR 364-NN had the highest $\delta^{15}N$ value which was significantly different ($P<0.05$) to all other lines (Table 5.4A). In general, plants of lines grown under drought had reduced leaf nitrogen. But irrespective of the growth condition used, the highest shoot %N in both treatments was found for the commercial cultivar PAN 185 which was significantly ($P<0.05$) different to all other lines under drought. Non-fixing lines, as expected, exhibited the lowest shoot %N under both growth conditions (Table 5.4A).

Although difference existed between bean lines for seed %N under both growth conditions, this difference was not significant ($P>0.05$) among the N-fixing lines under well-watered conditions. Commercial cultivar PAN 185 and the two non-fixing lines BAT 477-NN and DOR 364-NN had significantly ($P<0.05$) lower values than all other N-fixing lines (Table 5.4B). However, under drought the three bean lines BT_34 -1-1, BT_6-1-1, and BAT 477 had the highest %N and the non-fixing lines the lowest %N values (Table 5.4B).

Under well-watered growth conditions %Ndfa ranged from 47.6-63.5% with no significant ($P>0.05$) difference between N-fixing bean lines (data not shown). Under drought, bean lines significantly differed ($P<0.05$) for %Ndfa with BT_6-1-1, BT_34 -1-1, BT_51-1-1 and BAT 477 having higher values than all other lines (Table 5.4B). The amount of nitrogen fixed per plot (g/2.25 m^2) revealed that PAN 185, BAT 477, and BT_6-1-1 were best N-fixing (3.07-5.0 g/2.25 m^2) under well-watered conditions. However, under drought PAN 185 had the highest value followed by BAT 477, BT_51-1-1, and BT_34-1-1 (Table 5.4B).

At both water regimes, seed 15N/14N ($\delta^{15}N$) and shoot 15N/14N ($\delta^{15}N$) were significantly ($P<0.05$) and positively correlated (Table 5.5A and B). Also a significant positive relation ($P<0.05$) was found between, seed and shoot %N, fixed N/plot and shoot %N, %Ndfa and %N (shoot and seed), except for seed %N and shoot %N under drought which was not significant (Table 5.5A and B). Other parameters were negatively related to each other and a significant ($P<0.05$) relation was found between shoot %N with both shoot and seed 15N/14N, seed %N with both shoot and seed 15N/14N and %Ndfa with both shoot and seed 15N/14N.

Table 5.3 Nodule size of seven bean lines grown in the field under well-watered and drought conditions. Data represent mean ± SEM of four plants per plot (for twelve individual plants per line) after exposure for one month to drought, 30 days after planting.

Lines	**Nodule size (mm)**		
	Well-watered	**Drought**	**% reduction**
BT_6-1-1	3.09±0.24a	1.75±0.28c	43.37
BT_34-1-1	2.63±0.22abc	2.47±0.26ab	6.08
BT_51-1-1	2.69±0.27abc	2.00±0.29bc	25.65
BT_147-3	2.64±0.15abc	1.79±0.28bc	32.20
DOR 364	2.13±0.29c	1.67±0.18c	21.60
BAT 477	3.00±0.22a	2.44±0.19ab	18.67
PAN 185	2.93±0.26ab	2.87±0.26a	2.05
Significance	*	**	

Significance level was determined using ANOVA (** $P<0.001$) and (*$P<0.05$). Difference between treatment means was determined using the LSmeans Student's t-test. % reduction is the calculated reduction of nodule size from the difference of well-watered and drought. Means followed by the same letter within the column are not significantly different.

Table 5.4A Performance of nine bean lines for δ^{15}N and %N analysis for shoot and seed samples. The result is the mean of three replicates (each replicate from a composite sample of three plant samples) for each bean lines for each water regime.

Lines	**Shoot δ^{15}N**		**Seed δ^{15}N**		**Shoot %N**	
	Well-watered	**Drought**	**Well-watered**	**Drought**	**Well-watered**	**Drought**
<u>*N-fixing*</u>						
BT_6-1-1	-0.107±0.77bc	-0.671±0.8d	-1.630±0.15cde	-0.923±0.23bc	2.633±0.28ab	1.765±0.36bcde
BT_34-1-1	-1.454±0.67c	-0.373±0.22cd	-2.072±0.24ef	-0.615±0.61bc	2.458±0.32b	1.967±0.37bcd
BT_51-1-1	-1.304±0.43bc	0.377±0.04cd	-2.763±0.27f	-0.797±0.28bc	2.278±0.22b	2.116±0.21bc
BT_147-3	-0.762±0.68bc	1.463±0.34b	-0.850±0.33bc	-0.156±0.9abc	2.23±0.09b	1.828±0.1bcd
DOR 364	-1.447±0.28c	0.594±0.75bc	-1.195±0.26bcd	0.042±0.53ab	2.271±0.26b	1.685±0.29cde
BAT 477	-0.400±0.31bc	-0.535±0.48d	-1.852±0.24de	-0.815±0.13bc	2.680±0.17ab	2.169±0.18b
PAN 185	0.066±1.14abc	0.587±0.44bc	-0.458±0.04b	-1.531±0.48c	3.360±0.04a	2.715±0.33a
<u>*Non fixing*</u>						
DOR 364-NN	0.410±0.16ab	2.840±0.06a	1.139±0.04a	1.108±0.75a	1.442±0.06c	1.542±0.14de
BAT 477-NN	1.735±0.25a	1.650±0.04b	1.212±0.29a	0.971±0.1a	1.445±0.14c	1.339±0.30e
Significance	*	**	**	*	**	**

Significance level was determined using ANOVA (** *P<0.001* and, * *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.

Table 5.4B Performance of nine bean lines for seed %N, %Ndfa and Fixed N (g/m^2). The result is the mean ±SEM of three replicates (each replicate from a composite sample of three plant samples) for each bean lines for each water regime.

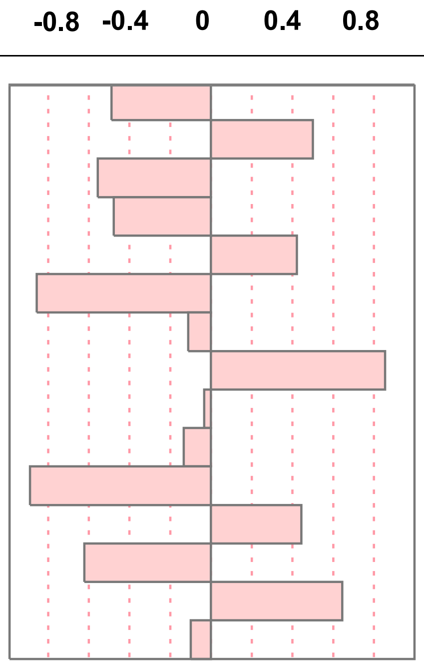
Lines	**Seed %N**		**%Ndfa**	**Fixed N / 2.25 (g/m^2)**	
	Well-watered	**Drought**	**Drought**	**Well-watered**	**Drought**
N-fixing					
BT_6-1-1	4.30±0.28a	4.29±0.36ab	57.54±2.2a	3.070±0.52ab	0.625±0.13c
BT_34-1-1	4.31±0.32a	4.55±0.37a	54.67±5.9ab	2.824±0.52bc	1.661±0.08b
BT_51-1-1	4.21±0.22a	3.64±0.21bc	47.46±2.7abc	1.836±0.06bc	1.880±0.11b
BT_147-3	3.73±0.09a	3.63±0.1bc	37.01±8.6c	1.085±0.24c	0.796±0.05c
DOR 364	3.79±0.26a	3.35±0.29c	45.37±5.1bc	2.875±0.27bc	0.306±0.18c
BAT 477	3.746±0.17a	4.37±0.18ab	56.24±1.3ab	3.263±0.12ab	2.298±0.35b
PAN 185	3.04±0.04b	3.46±0.33c	45.4±4.7bc	4.950±1.5a	3.968±0.55a
Non-fixing					
DOR 364-NN	2.46±0.06b	3.23±0.14c	na	na	na
BAT 477-NN	2.71±0.14b	3.34±0.3c	na	na	na
Significance	**	*	*	*	**

na= not applicable (since they are non-fixing lines), %Ndfa= percentage of legume N derived from the atmosphere.

Significance level was determined using ANOVA (** $P<0.001$, and *$P<0.05$) 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

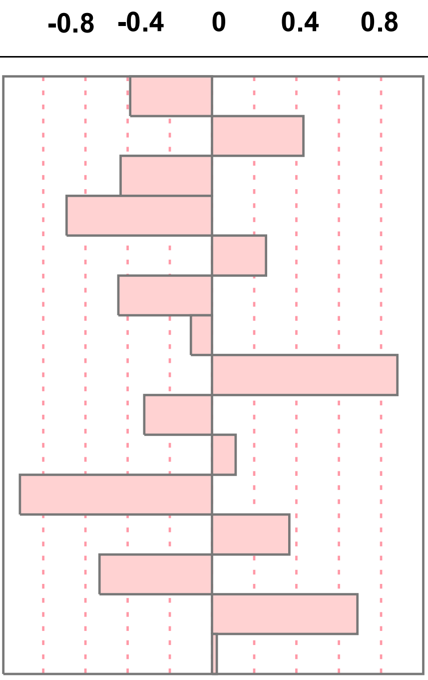
Table 5.5 (A) Association between shoot and seed δ^{15}N (d15N/14N), % N, fixed N, and % Ndfa for seven common bean lines. Data obtained from pooled data of three replicates (each replicate from a composite sample of three plant samples) for each bean lines for δ^{15}N, % N and from the computation of 2.25 m^2 for fixed N, and % Ndfa for plants grown under well-watered growth conditions.

Traits		**r-value**	**P-value**
Shoot N%	Shoot d15N/14N	-0.4921	**
Seed 15N/14N	Shoot d15N/14N	0.5077	**
Seed 15N/14N	Shoot N%	-0.5635	**
Seed %N	Shoot d15N/14N	-0.4778	*
Seed %N	Shoot N%	0.4255	*
Seed %N	Seed 15N/14N	-0.8657	**
Fixed N/ plot	Shoot d15N/14N	-0.1156	ns
Fixed N/ plot	Shoot N%	0.8597	**
Fixed N/ plot	Seed 15N/14N	-0.0312	ns
Fixed N/ plot	Seed %N	-0.1351	ns
%Ndf	Shoot d15N/14N	-0.8983	**
%Ndf	Shoot N%	0.4521	*
%Ndf	Seed 15N/14N	-0.6307	**
%Ndf	Seed %N	0.6515	**
%Ndf	Fixed N/ plot	-0.1013	ns



r = Pearson's correlation coefficient,* indicates the correlation is significantly different *(P<0.05),* ** indicates the correlation is highly significant (P<0.01) and ns indicates the correlation is non-significant (*P>0.05*). Bars showing the r- value of the association.

Table 5.5 (B) Association between shoot and seed $\delta^{15}N$ (d15N/14N), % N, fixed N, and % Ndfa for seven common bean lines. Data obtained from pooled data of three replicates (each replicate from a composite sample of three plant samples) for each bean lines for δ15N, % N and from the computation of 2.25 m^2 for fixed N, and % Ndfa for plants grown under water-limited growth condition.



Traits		**r-value**	**P-value**
Shoot N%	Shoot d15N/14N	-0.3929	*
Seed 15N/14N	Shoot d15N/14N	0.4357	*
Seed 15N/14N	Shoot N%	-0.4328	*
Seed %N	Shoot d15N/14N	-0.6964	**
Seed %N	Shoot N%	0.2546	ns
Seed %N	Seed 15N/14N	-0.4457	*
Fixed N/ plot	Shoot d15N/14N	-0.1013	ns
Fixed N/ plot	Shoot N%	0.8857	**
Fixed N/ plot	Seed 15N/14N	-0.3221	ns
Fixed N/ plot	Seed %N	0.1117	ns
%Ndf	Shoot d15N/14N	-0.9125	**
%Ndf	Shoot N%	0.3637	*
%Ndf	Seed 15N/14N	-0.5414	**
%Ndf	Seed %N	0.6958	**
%Ndf	Fixed N/ plot	0.0201	ns

r = Pearson's correlation coefficient,* indicates the correlation is significantly different *(P<0.05),* ** indicates the correlation is highly significant (P<0.01) and ns indicates the correlation is non-significant (*P>0.05*). Bars showing the r- value of the association.

5.4.2.1 *Relation of $\delta^{15}N$ and carbon isotope discrimination, as well as with root and productivity traits*

Relation of root morphological traits (root length, area and volume) with shoot $\delta^{15}N$ was significantly ($P<0.05$, negatively) at both water regimes except for root length under well-watered conditions. Additional significant negative relations ($P<0.05$) were found between shoot $\delta^{15}N$ with architectural traits of 1st whorl angle, basal root number, basal and adventitious root branching density under drought. However, for well-watered conditions, a significant negative ($P<0.05$) association of $\delta^{15}N$ with root architectural traits was only found for basal root number (Table 5.7). Shoot CID was significantly ($P<0.05$) negatively related with root morphological traits (root length, area and volume) and root architectural traits (basal root number as well as with 1st and 2nd whorl angles) under drought condition (Table 5.7).

Under both growth conditions, shoot and seed $\delta^{15}N$ was significantly ($P<0.05$), positively related with both shoot and seed CID except for shoot CID with shoot $\delta^{15}N$ and seed $\delta^{15}N$ under well-watered and drought conditions respectively(Table 5.6). Further, significant negative relationship ($P<0.05$) was found under both water regimes for %N with CID (shoot and seed) except for shoot %N with seed CID under drought (Table 5.6).

When the relation of $\delta^{15}N$ with productivity parameters (seed yield and pod harvest index) was determined, the relation between $\delta^{15}N$ and PHI was significant under both well-watered ($r^2=0.54$, $P<0.0001$) and drought conditions ($r^2=0.56$, $P<0.0001$) (Figure 5.1). Further, a significant relation under drought existed between seed yield and shoot $\delta^{15}N$ ($R^2=0.42$ and $P<0.0003$) and under well-watered condition between seed yield and seed $\delta^{15}N$ ($R^2=0.53$ and $P<0.001$) (Figure 5.2).

Table 5.6 The association of shoot and seed CID with shoot and seed $\delta^{15}N$ as well as %N for seven common bean lines grown under well-watered and drought conditions. Data were obtained from pooled data of three replicates (each replicate from a composite sample of three plant samples) for each bean line and water regime.

Trait	**Shoot CID**		**Seed CID**	
	Well-watered	**Drought**	**Well-watered**	**Drought**
Shoot $\delta^{15}N$	0.251 Ns	0.548 *	0.475 **	0.548 **
Shoot% N	-0.357 **	-0.627 **	-0.556 **	-0.230 ns
Seed δ15N	0.583 **	0.388 ns	0.700 **	0.527 *
Seed %N	-0.543 **	-0.446 *	-0.622 **	-0.561 **

r = Pearson's correlation coefficient, * indicates the correlation is significantly different *($P<0.05$),* ** indicates the correlation is highly significant ($P<0.01$) and ns indicates the correlation is non-significant (*$P>0.05$*).

Table 5.7 Association of root morphological and architectural traits with shoots δ^{15}N and shoots CID. Data for all traits represent correlation of overall means of nine bean lines during the experimental period under well-watered or drought conditions.

		Well-watered		**Drought**	
	Trait	**r**	**P-value**	**r**	**P-value**
Shoot δ^{15}N	Root length	-0.510	0.0671	-0.609	0.0499*
	Root area	-0.587	0.0424*	-0.653	0.0439*
	Root volume	-0.704	0.0358*	-0.667	0.0037**
	1st whorl angle	-0.320	0.2176	-0.664	0.0159*
	2nd whorl angle	-0.431	0.3085	-0.222	0.7001
	Basal root number	-0.771	0.0237*	-0.701	0.047*
	Basal root bran. density	-0.483	0.3317	-0.228	0.0438*
	Tap root bran. density	-0.264	0.4328	-0.019	0.9661
	Adv. root bran. density	-0.248	0.9319	-0.671	0.0159*
	Adv. root width	0.344	0.0992	0.197	0.8647
Shoot δ^{13}C	Root length	-0.337	0.4064	-0.467	0.0475*
	Root area	-0.351	0.5457	-0.537	0.0469*
	Root volume	-0.394	0.4328	-0.617	0.0358*
	1st whorl angle	-0.436	0.3743	-0.786	0.0005**
	2nd whorl angle	-0.369	0.5457	-0.577	0.0199*
	Basal root number	-0.424	0.5003	-0.732	0.0079**
	Basal root bran. density	0.052	0.8312	0.286	0.4328
	Tap root bran. density	0.189	0.6682	0.110	0.5755
	Adv. root bran. density	-0.181	0.6354	-0.383	0.2646
	Adv. root width	0.172	0.7980	0.006	0.8647

r = Pearson's correlation coefficient, * indicates the correlation is significantly different *($P<0.05$),* ** indicates the correlation is highly significant ($P<0.01$) and ns indicates the correlation is non-significant (*$P>0.05$*). Adv= adventitious, bran. =branching.

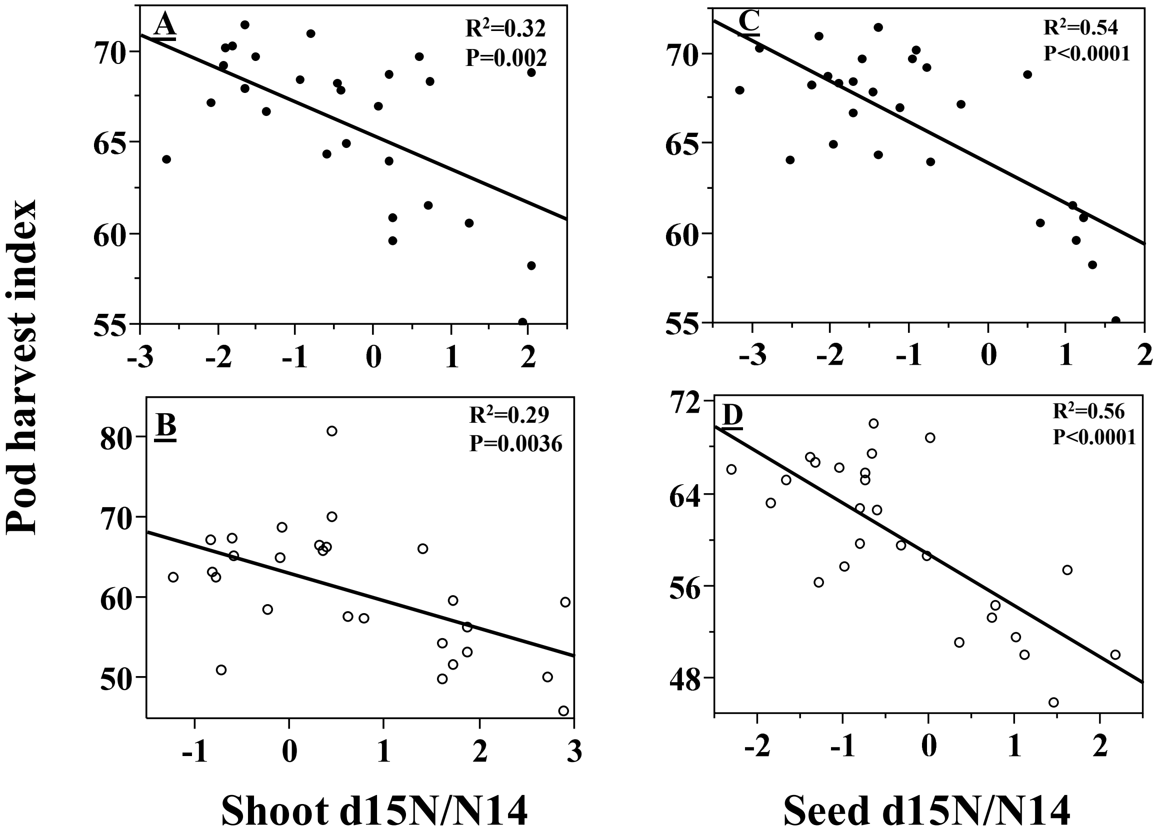


Figure 5.1 Relationship between shoot (A and B) and seed (C and D) d15N/14N (δ15N) and pod harvest index (PHI) of nine common bean lines. Data were obtained from one row per plot at harvest for PHI and analysis for δ^{15}N shoot and seed sample from plants grown under well-watered (A and C) and drought conditions (B and D) from three replicates (each replicate from a composite sample of three plant samples) for each bean lines for each water regime.

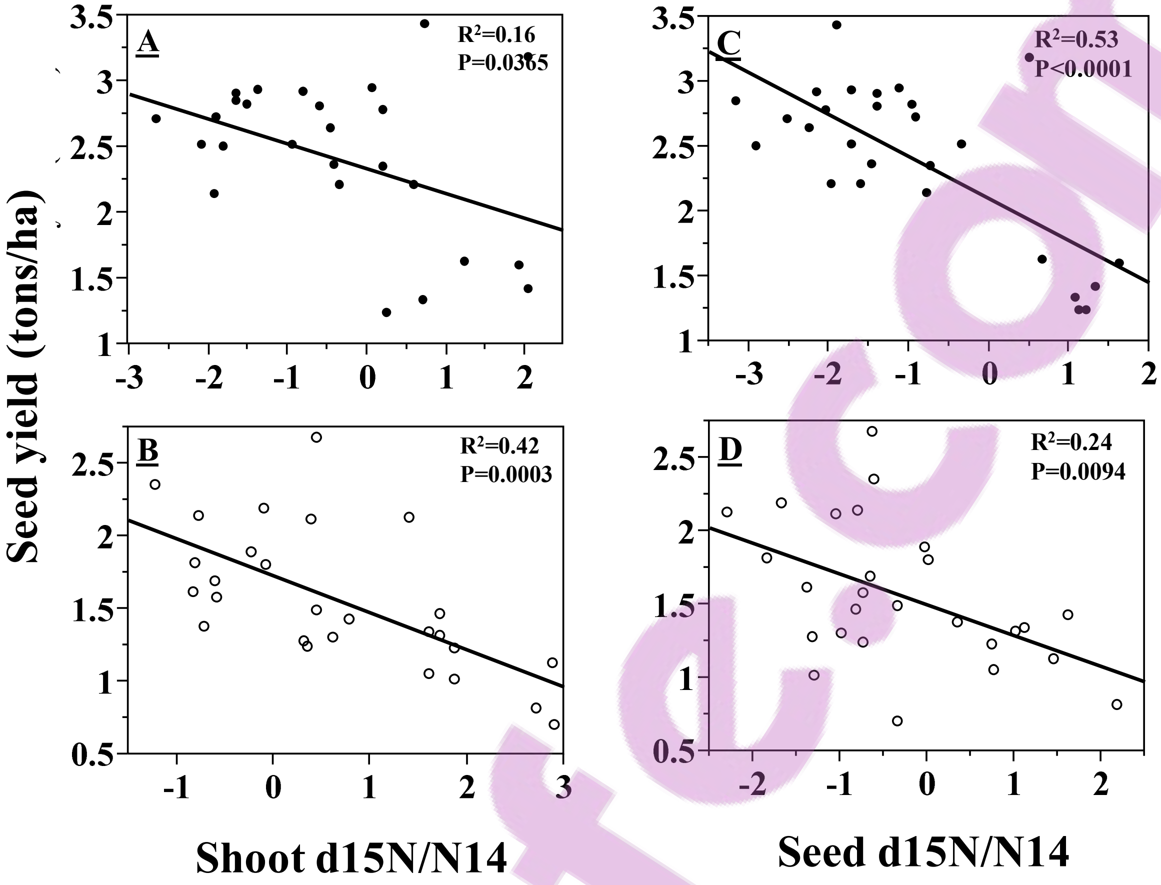


Figure 5.2 Association between shoot (A and B) and seed (C and D) δ^{15}N and seed yield of nine common bean lines. Data of seed yield were obtained from the harvest of two rows per plot and analysis for δ^{15}N seed sample per plot from plants grown under well-watered (A and C) and water-limited (B and D) growth condition from three replicates (each replicate from a composite sample of three plant samples) for each bean lines for each water regime.

5.5 Discussion

In this part of the study the question was asked if there is a direct relationship between the carbon isotope discrimination as well as ^{15}N natural abundance with seed yield, root traits and nodule performance under well-watered and drought conditions. Results of the study clearly show existence of a direct relationship between both isotopic ratio and seed yield, pod harvesting index, root traits and nodule performance.

This study has also shown that N-fixing bean lines had superior performance for CID independent of growth conditions when compared to non-N fixing lines with lines BT_6-1-1, BAT 477, BT_34-1-1 and BT _51-1-1 as well as commercial cultivar PAN 185 having the highest WUE (lower value of CID) for shoot and seed CID when compared to all other lines studied. This demonstrates an important relationship between nitrogen fixation and metabolism, and carbon fixation (WUE), and is in agreement with the suggestion made by Raven and Farquhar (1990) that, change of CID is due to a change in nitrogen availability by C_3 plants. Further, values found for $\delta^{13}C$ were also in agreement with values reported by Harmon (1957) and Troughton et al. (1974) for C_3 plants with $\delta^{13}C$ ranging from -20 to -32 ‰ (CID, 12.24 to 24.79‰). In this study, CID for shoots ranged from CID, 21.38 to 22.44‰ under well-watered conditions and from 20.63 to 21.68‰ under drought. For seed CID, a higher variation was found under well-watered conditions 18.33 to 21.69‰ than under drought 18.80 to 20.49‰. This result is in agreement with the values reported for wheat seeds (Farquhar and Richards, 1984), tomato leaf (Martin and Thorstenson, 1988), for beans shoot (Zacharisen et al., 1999) and shoot and seed of wheat (Shaheen and Hood-Nowotny, 2005). Higher discrimination was observed for shoot than seed plant samples, this has been also in agreement in earlier studies for several legumes by Yoneyama and Ohtani (1983). As it has been proposed by Hubick et al. (1986) it might be due to the lower fraction of carboxylation involving phosphoenolpyruvate carboxylase (PEPCase) in leaves than seeds. This is because, PEPCase has more affinity to ^{13}C than carboxylation by RubP carboxylase (O'Leary, 1981). Furthermore, the difference of CID value between shoot and seed might be partly due to the difference in the source of carbon for these plant parts. The shoot carbon isotope measurement indicates the carbon obtained from photosynthetic sugar whereas in the seed the starch reserve that has been assimilated from the plant (Deleens et al., 1994). Bean lines with higher carbon isotope discrimination had a higher %C under both growth conditions. This confirms previous results by Hubick and Farquhar (1989) in barley where the carbon content of the dry matter highly correlated with CID. This correlation might be due to the influence CID on long-term carbon dioxide fixation throughout the growth period of the plant (Evans et al., 1986).

This study has also extended application of the CID technique to beans. Further, the importance of WUE, measured as CID, and HI estimated by PHI, as indicators for plant performance was proofed the postulated made by Passioura (1996) Y=WU x WUE x HI, where ,Y is yield, and WU is water use. However, a low shoot CID value (high WUE) under drought measured for line DOR 364, but associated with lower yield, suggests that CID should not be solely measured as a performance indicator. A significant relation of shoot and seed CID was further found with productivity traits (PHI and seed yield) and morphological (root length, area and volume) as well as architectural (basal root number, and 1st as well as 2nd whorl angles) root traits, especially under drought. This indicates that bean lines with enhanced WUE, such as BT_6-1-1, BAT 477 and BT_34-1-1, also maintain better stomatal conductance with concurrent high carbon fixation and ultimately better productivity and performance under drought. The relationship of PHI and $\delta^{15}N$ (SNF) was better for seed than shoots. PHI reflects movement of C to pods. If fixed N is also mobilized preferentially to pods, perhaps this correlation reflects a general tendency for resource mobilization during the grain fill period.

The finding the negative association of CID with productivity traits was consistent with the previous reports at non-stress condition in wheat (Khazaei et al., 2009; Rebetzke et al., 2006) as well as under water stress condition in barley (Craufurd et al., 1991) and other C_3 plants (Brugnoli and Farquhar, 2000; Craufurd et al., 1991). This result suggest that higher carbon isotope discrimination (lower CID value) were related to higher photosynthetic CO_2 assimilation which ultimately contributed to higher seed yield as it has been also proposed by Ehleringer (1990). This argument is also further supported by better SPAD value for the bean lines performing well under drought with higher WUE (low CID*)*. Corresponding to this report, existence of a linear and positive correlation of SPAD with WUE and photosynthetic CO_2 assimilation has been found by Evans (1983) and Kapotis et al., (2003). There is further a strong positive association reported for chlorophyll content and SPAD in several crops (Kapotis et al., 2003; Uddling et al., 2007; Yamamoto et al., 2002).

This study also revealed existence of a significant positive, relationship between CID and nitrogen fixation which is a merit association since the lower value for both isotope analysis mean higher WUE and SNF. Further, significant negative relations between CID and shoot and seed %N also support the relationship of CID and $\delta^{15}N$ (SNF). A complementation of carbon fixation with nitrogen fixation as well as nitrogen metabolism has been previously reported for lentil by Knight et al., (1993). Bean lines with higher WUE (lower CID) also had higher %N in their shoot and seed, but lower $\delta^{13}N$, in contrast, non-fixing lines had a low WUE (higher CID value) and %N but higher $\delta^{15}N$ values. The CID value is affected by the amount of nitrogen and its metabolism in plants due to the requirement of carbon atoms from CO_2 assimilation in organic nitrogen compounds (Raven and Farquhar, 1990). Further, a negative effect of drought on both carbon assimilation (WUE) and nitrogen fixation, as found in this study by measuring CID, %N, %C and $\delta^{15}N$, has also been reported by other researchers (Djekoun and Planchon, 1990; King and Purcell, 2006). Overall, data indicate a strong relationship between nitrogen fixing efficiency and WUE, measured as CID, which has not been reported so far for beans but has previously been reported for rice regarding association of CID with nitrogen (Kondo et al., 2004). Furthermore, although they should be drawing on the same soil pool of nitrogen, non-fixing lines (DOR 364-NN and BAT 477-NN) presented significantly different lower WUE (higher CID) and SNF (^{15}N), this might be due to, they have distinct root systems and probably are exploring different segments of the soil and because of their retarded growth due to deficiency of nitrogen since they are not fixing N.

In conclusion, this study has shown that nitrogen fixing lines performed better than non-fixing lines for PHI and yield with lines BAT 477, BT_51-1-1 and BT_34-1-1 as well as commercial cultivar (PAN 185) outperforming all other lines under water-limited condition. Superior performance of the above lines is in agreement with evaluation of these bean lines under greenhouse conditions, where these lines also outperformed all other tested lines. Further, for the first time this study has shown that there is a direct relationship between both carbon isotope discrimination - (WUE) and nitrogen fixation (^{15}N) and root morphological and architectural traits (root length, area and volume, basal root number, 1st as well as 2nd whorl angles). This is beneficial to maintain the water status of the plant independent of the environmental conditions particularly under drought. Therefore root traits might be used as easily measurable markers for bean performance under drought. Also, for the first time this study has shown that there is a direct relationship between carbon isotope discrimination - (WUE) and nitrogen fixation (^{15}N) as well as maintaining nodule size. This would be beneficial for extending the life time of bean nodules. Maintaining nodule size could be further a marker for bean productivity under drought. A further novel result of this study was that that carbon isotope discrimination - (WUE) and nitrogen fixation (^{15}N) is directly related in beans with PHI and seed yield. Further, the existence of non-significant difference for SNF traits and CID for the interaction of lines vs. water treatment suggests as the bean inbred lines performance were consistent for these parameters across the two water regimes. Moreover, since these traits have also associated with seed yield, the use of these traits will provide good selection criteria for been germplasm.

CHAPTER SIX

ADAPTATION OF SOYBEAN CULTIVARS TO DROUGHT STRESS UNDER FIELD CONDITIONS: ROLE OF ROOT ARCHITECTURE, BIOMASS PARTITIONING, WATER USE EFFICIENCY AND SYMBIOTIC NITROGEN FIXATION

6.1 Abstract

Field experiments were conducted using three soybean cultivars (Jackson, A5409RG, Prima 2000) which were also used in a previous greenhouse study under well-watered and drought stress condition. The main objective was to evaluate root and shoot traits under drought stress to determine the physiological basis of differences in growth and seed yield. Drought stress was induced at one month after establishment and measurements were made at three growth stages (flowering, mid-pod filling and at harvest). Measurements, included root architectural and morphological traits, biomass partitioning, leaf chlorophyll content, water use efficiency (WUE) and symbiotic nitrogen fixation (SNF). WUE and SNF were measured as carbon isotope discrimination and ^{15}N natural abundance ($\delta^{15}N$), respectively. Prima 2000 and Jackson performed better under drought when root morphology and architectural traits, CID and $\delta^{15}N$ were used as performance parameters. Jackson as an early maturing cultivar was superior to other two cultivars in partitioning a greater proportion of biomass to seed under drought stress. However, higher values of total dry mass as well as seed yield under drought were observed with the longer maturing Prima 2000 when compared to the other two soybean cultivars. Among the three cultivars, longer maturing and transgenic A5409RG was least adapted to drought stress. There was also a significant correlation of CID (WUE) with $\delta^{15}N$ (SNF) as well as root traits and seed yield under drought. Results also indicate that lower values of CID under drought could contribute to higher CO_2 assimilation resulting in better N_2 fixation. Use of root architectural traits, such as diameter and branching density, and morphological traits, such as root length, surface area and volume, might be useful not only to evaluate genotypic differences in response to drought but also to improve genetic adaptation of soybean to drought stress.

6.2 Introduction

Genetic variability exists in the soybean germplasm for root architectural traits, such as root angle (Zhao et al., 2004) root diameter and rooting depth (Ao et al., 2010), and morphology traits such as root length (Zhao et al., 2004), root volume, area, and length (Ao et al., 2010). Further, it was also shown that the differential performance of soybean cultivars was related to root length, density and dry mass that contributes to improved water absorption under drought (Garay and Wilhelm, 1982). The ability of the plant to extract greater amounts of available soil water under drought conditions through the deep root architecture has been shown to contribute to improved growth and seed yield (Garay and Wilhelm, 1982; Zhao et al., 2004). However, the methodology to evaluate root architecture under field conditions needs to be improved and its contribution to performance of soybean needs to be defined. Therefore, more detailed information is needed on root architecture and morphology traits.

Since measurement of water use efficiency (WUE) using carbon isotope discrimination provides information about the longer-term plant performance associated with CO_2 fixation, CID can be used as a surrogate for WUE. Previous results indicated that carbon is incorporated into the plant tissue, transported and metabolized for a substantial time during the entire growth period of the plant (Evans et al., 1986; Johnson et al., 1990; Shaheen and Hood-Nowotny, 2005). Therefore, the CID technique has been used in several crops for performance evaluation under abiotic stress to demonstrate a possible relation between photosynthetic assimilation and water utilization (Farquhar et al., 1989). Stresses investigated include drought and salinity in wheat (Shaheen and Hood-Nowotny, 2005) and waterlogging and salinity in clover and puccinellia (Mostajeran and

Rengel, 2007). Although little information is available on the use of CID in soybean improvements, the potential of CID as a performance measurement for WUE in soybean has been suggested by Kumarasinghe et al. (1992) and White et al. (1996). Ultimately, using CID for performance evaluation in the field might help to better understand the response of soybean cultivars to drought under field conditions. For field-grown legumes, measurement of ^{15}N natural abundance is one of the widely used methods to assess N fixation ability. The principle behind this technique is that the concentration of ^{15}N in biologically fixed N is lower than that of N from other sources and is based on small difference in ^{15}N concentration (Shearer and Kohl, 1986). The importance of the use of ^{15}N abundance for measurement of SNF performance in soybean field experiments has been proposed (George T. et al., 1996; Kumarasinghe et al., 1992). Using non-fixing legumes or cereals (weeds) for studying natural abundance of nitrogen in legumes is a common procedure and has been used in soybean and clover with ryegrass and marigold (Kohi et al., 1980) as well as in pea with barley (Holdensen et al., 2007) and when ten different annual legumes were compared and wheat was used as a control in field studies (Unkovich and Pate, 2000).

The hypothesis of this study was, differential variation in root architecture and morphology traits, including shoot and productivity markers for soybean cultivar grown under field exists and these genetic differences can help for performance evaluation under water-limited condition. Further, it has been also hypothesized that similar in common bean water use efficiency as measured by carbon isotope discrimination as well as symbiotic nitrogen fixation determined by ^{15}N natural abundance is associated with productivity as well as root traits to use as selection marker under drought studies. Moreover, this study was also aimed to verify if performance of soybean cultivars for drought stress under filed condition will be comparable as the output found under control condition and plant traits used for performance evaluation would be the similar with common bean.

6.3 Materials and methods

6.3.1 Plant material and Experimental procedure

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). Other experimental area description as indicated at chapter four. Soybean (*Glycine max* L. Merr.) cultivars which exhibit different background were used in this study: A5409RG, a glyphosate resistant transgenic cultivar, Prima 2000, a commercial cultivar grown in South Africa, and Jackson, considered to be a drought-tolerant cultivar (Chen et al., 2007; Sall and Sinclair, 1991). Since a non-fixing soybean cultivar was not available as a reference/control for isotope studies, two non-fixing common bean lines (DOR 364-NN and BAT 477-NN) were used as controls. The two lines were grown in the same field using an identical experimental design.

Before the commencement of the experiment, a soil analysis for both macro and micro-nutrients was conducted by Alpha Agric PLC soil analysis laboratory, Nylstroom, South Africa. Based on the results, 4 kg/ha boron, 1 kg/ha zinc sulfate and 25 kg/ha potassium sulfate were applied. Before land preparation 3L/ha of Roundup, a systemic, broad-spectrum herbicide were applied to kill all the weeds on the field. Before planting the pre-emergence herbicides Unimoc EC

(800ml/ha) and Imazethaphyr (400ml/ha) were also sprayed to control both grasses and broadleaf weeds. After planting, hand-weeding was performed as needed. The nematicide oxadate (3L/ha) was applied to prevent nematode infestation for up to a month after planting.

6.3.3 Planting and experimental layout

The experiment was conducted in a randomized complete block design with two water regimes, with well watered and water-limited. Each cultivar was planted in five rows with the spacing of 75 cm between rows and 10 cm between plants. Row length was 4 m and plot size was 12 m^2. The central three rows were used for data collection, while the two outer rows served as borders. One seed per hole was planted at 5 cm depth using a Jab planter which is specially designed to plant with uniform depth.

Before the initiation of drought stress, all the treatments were grown with adequate water supply by applying 8 mm water/day using pivot sprinkler irrigation. Drought stress was started 30 days after planting by turning off the sprinkler nozzles. Moisture stress was applied for 28 days, although rain fell on the 7th, 19th and 26th days after planting with a total of 34 mm after which data collection started.

6.3.4 Measured parameters

6.3.4.1 Chlorophyll content

Three plants per plot of each variety (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 non-destructively using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing, Inc., Japan) and chlorophyll content of three individual SPAD chlorophyll meter readings (SCMR) per plot were averaged.

6.3.4.2 Root architecture

Root architectural measurements were made for the two main root types (Figure 6.1) of soybeans after one month of drought exposure. Three plants per plot and per replication (nine plants per cultivar) for each water regimes were sampled using the “Shovelomics”(Lynch, 2011; Trachsel et al., 2011) technique. Subsequently, tap and lateral root thickness (diameter) was measured by multiple measurements 2 cm away from the origin of these roots or attachment using an electronic digital caliper 5HA 1890 Model (Omni- Tech). The branching density of tap and lateral root was determined by counting the lateral root/root hairs emerging within 2 cm root segment of the tap root and for three randomly selected lateral roots.

6.3.4.3 Root morphology analysis

After one month of exposure of plants to drought, three soil cores per plot were taken in each water regime for analysis of the root morphology. The steel corer lined with a plastic tube (60 cm length and 42 mm diameter) (Giddings Machine Company Inc, USA) was driven into the soil in between two plants. Upon extracting the core, roots were washed out of the soil, scanned with a root scanner (Epson Perfection V 700 Photo /V 750 Pro (Seiko Epson Corporation 2005) and the root images were analyzed using the winRHIZO 2008a program (Regent Instruments Canada Inc., Canada) to determine root morphology.

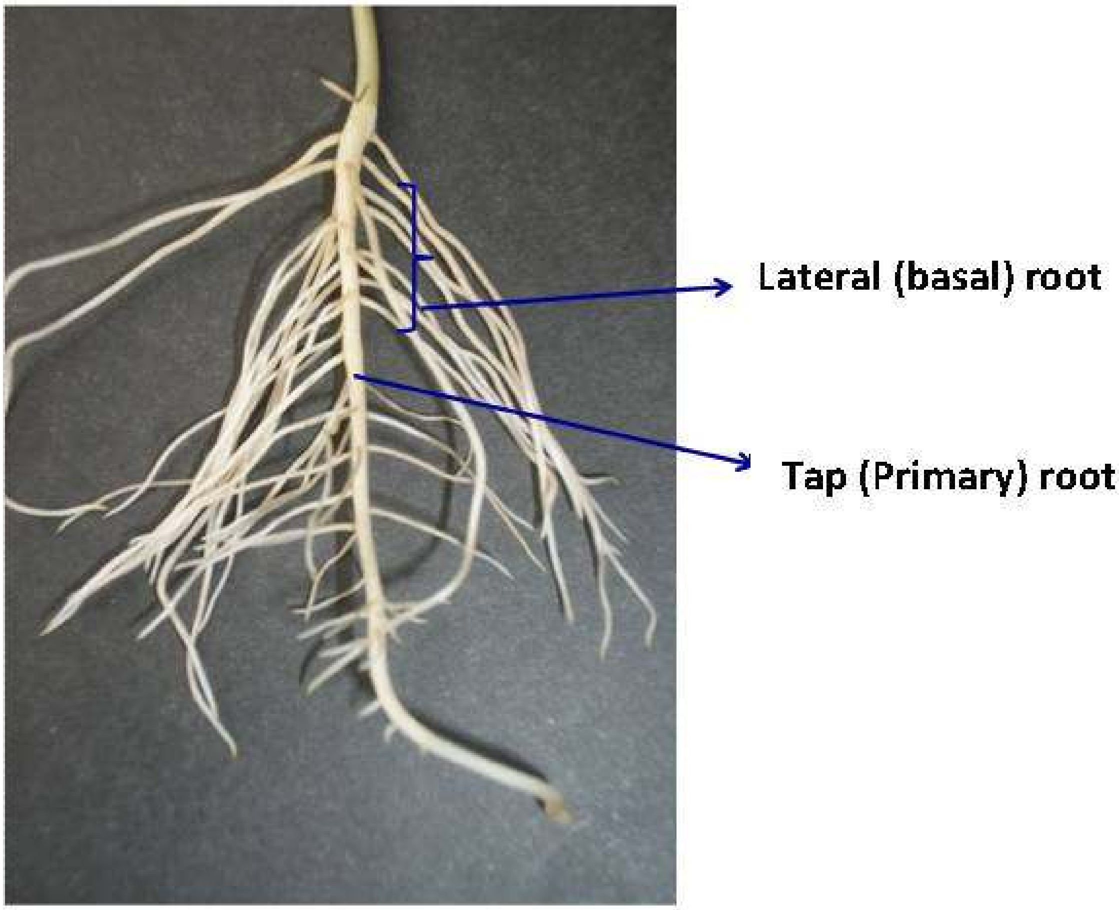


Figure 6.1 Schematic representation of soybean root system architecture.

6.3.4.4 Biomass partitioning and seed yield measurement

At flowering and mid-pod filling stage six representative individual plants per plot for each water regime were harvested and plant parts were separated into leaves, stems and pods (at mid-pod filling stage). Dry mass of plant parts was determined after oven drying at 60^0C for 48 h (TERM-O-MAT LABOTEC, South Africa). For determining seed yield of each plot, two rows of 3 m length (2.25 m^2 area) were used, after discarding a border of 0.5 m on both extremes of the rows. Yield per plot and per hectare was computed. Furthermore, 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 48 h and the dry mass was determined. Data were used to calculate the pod harvest index (PHI): (seed mass)/ (seed mass + pod wall mass).

6.3.4.5 *Nodule size, carbon isotope discrimination and ^{15}N natural abundance*

Root nodule size was determined by placing the root nodules on a board with a sketch of the diameter (mm) of nodules. For analysis of CID and ^{15}N natural abundance, the plants samples previously used dry mass determination of above ground parts (both leaf and stem) were ground to fine powder using a grinder (A 11 basic Analytical Mill, IKA® Works, Inc, Germany). The three plants per plot were bulked and ground to make up one replicate, and three replications were used. The samples were analyzed using Isotope ratio Mass Spectrometer (Thermo electron, Bremen, Germany) at Cape Town University, Department Archaeology. Carbon isotope discrimination was calculated from δ^{13}C of each plant sample (Farquhar et al., 1989).

Natural abundance of $\delta^{15}N$ was calculated using the formula previously reported (Shearer and Kohl, 1986; Unkovich et al., 1994; Valles-De La Mora et al., 2003). Furthermore, percentage of the nitrogen fixed by the legumes from the atmosphere was calculated as described before (Bergersen and Turner, 1983; Shearer and Kohl, 1986) (Peoples et al., 2009). The equations for calculation for CID, natural abundance of $\delta^{15}N$ and other SNF parameters is as described in chapter five at section 5.3.2.2.

6.3.5 Statistical analysis

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

6.4 Results

6.4.1 Chlorophyll content

SPAD chlorophyll meter readings (SCMR) of all cultivars were similar at the start of the experiment and for well-watered treatment after three weeks of drought stress measurement were (40-42 SCMR). Relative to the non-stress treatments, drought stress significantly reduced the chlorophyll content for cultivar A5409RG (10%), however, the reduction for other two cultivars was low (data not shown). Accordingly, under drought stress, Prima 2000 had significantly higher ($P<0.05$) chlorophyll content than A5409RG (Figure 6.2).

6.4.2 Root architecture and morphology

Significant interactions were revealed for water treatment and cultivar for two ways ANOVA analysis for root morphology traits (Appendix 9). However, none of root architectural traits were shown significant difference for water level vs. cultivar interaction (data not shown). For examining the complete story the main effect of root performance traits further evaluated for one way analysis of variance and treatment comparison. Accordingly, under well-watered conditions, all the root morphology traits (root length, area, volume, number of root tips and average diameter) were not significantly different between tested cultivars (Table 6.1). On the other hand, under drought conditions marked significant differences ($P<0.05$) were observed among the cultivars for the root morphology parameters except average root diameter. Drought significantly enhanced parameters of root morphology by 57, 36, 27, and 59% in root length, area, volume and number of root tips, respectively compared to well-watered condition. Prima 2000 and Jackson had significantly enhanced ($P<0.05$) root elongation, surface area and volume, relative to the A5409RG and these two cultivars had higher root length (up to 60%) and a 2 to 3-fold higher root total surface area and root volume than A5409RG which actually displayed a decrease in all root parameters (Table 6.1).

Regardless of the water regime, Jackson exhibited significantly higher ($P<0.05$) tap root diameter than the other two cultivars (Table 6.2). However, lateral root diameter (thickness) was significantly higher ($P<0.05$) for cultivar A5409RG under well-watered and drought conditions compared to Jackson and Prima (Table 6.2). Although moisture stress enhanced branching density of both tap and lateral root in all three cultivars, branching density differed among cultivars in the drought treatment. Relative to well watered treatment drought stress increased tap root branching by 29% 53%, and 57% for A5409RG, Jackson, and Prima 2000 respectively. Likewise, lateral root branching raise by 42%, 76%, 67% for A5409RG, Jackson, and Prima 2000 respectively (data not shown). These result and the output at Figures 6.3 and 6.4 shows, both under well-watered and drought conditions, Jackson and Prima 2000 had a higher tap and lateral root branching density when compared to A5409RG. Compared to previous computer simulation of root systems in soybean (Zhao et al., 2004) (Figure 6.5). A5409RG, Jackson and Prima 2000 exhibited shallow, deep and intermediate root architecture, respectively.

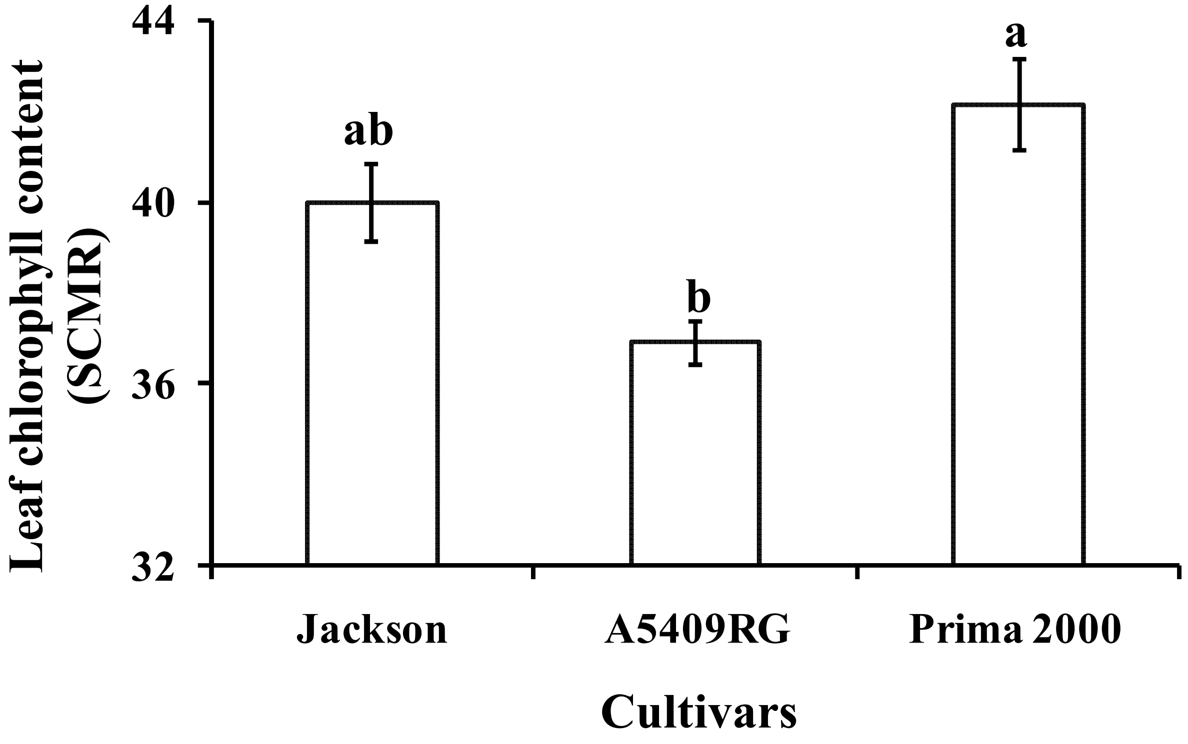


Figure 6.2 Leaf chlorophyll content (SCMR) of three soybean lines measured after three weeks of drought stress (drought block). Results are means ± SEM of three plants per plot and three repetitions. Means with the same letter above the bars are not significantly different as tested by LSmeans Student's t-test (P=0.05).

Table 6.1 Performance of root morphology traits of three soybean cultivars under well-watered and drought growth conditions. The root image was taken by a root scanner and analysis was made by using the winRHIZO 2008a software after 28 days of drought.

Cultivars	**Root length (cm)**	**Surface area (cm^2)**	**Root volume (cm^3)**	**Root tip number**	**Average diameter (mm)**
Well-watered					
A-5409RG	56.61±10.08	8.34±1.27	0.10±0.016	180.61±21.42	0.49±0.032
Jackson	56.91±11.72	8.05±1.48	0.09±0.018	190.50±24.89	0.46±0.037
Prima 2000	51.55±11.39	6.50±1.43	0.07±0.017	168.75±24.19	0.43±0.036
Significance	ns	ns	ns	Ns	ns
Drought					
A-5409RG	41.01±0.38b	4.74±2.31b	0.05± 0.03b	177.79±58.09b	0.40±0.068
Jackson	98.56±0.39a	11.05±1.79a	0.12±0.02a	301.44±44.99ab	0.48±0.052
Prima 2000	120.46±0.39a	15.45±1.83a	0.16±0.02a	377.79±45.92a	0.54±0.054
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 three replicates for each treatment using soil cores up to 60 cm soil depth.

Table 6.2 The performance of three soybean cultivars using mean separation for root architecture traits of tap and lateral root diameter (thickness) under well-watered and drought condition

Cultivars	**Tap root diameter (mm)**		**Lateral root diameter (mm)**	
	Well- watered	**Drought**	**Well- watered**	**Drought**
A-5409RG	2.53±0.15c	2.35±0.40c	4.14±0.33a	4.21±0.12a
Jackson	4.48±0.46a	4.34±0.25a	2.52±0.17b	2.15±1.13b
Prima 2000	3.78±0.12b	3. 63±0.51b	2.46±0.13b	2.23±0.16b
Significance	**	**	**	**

Significance level was determined using ANOVA (**P<0.001) 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 28 days of drought.

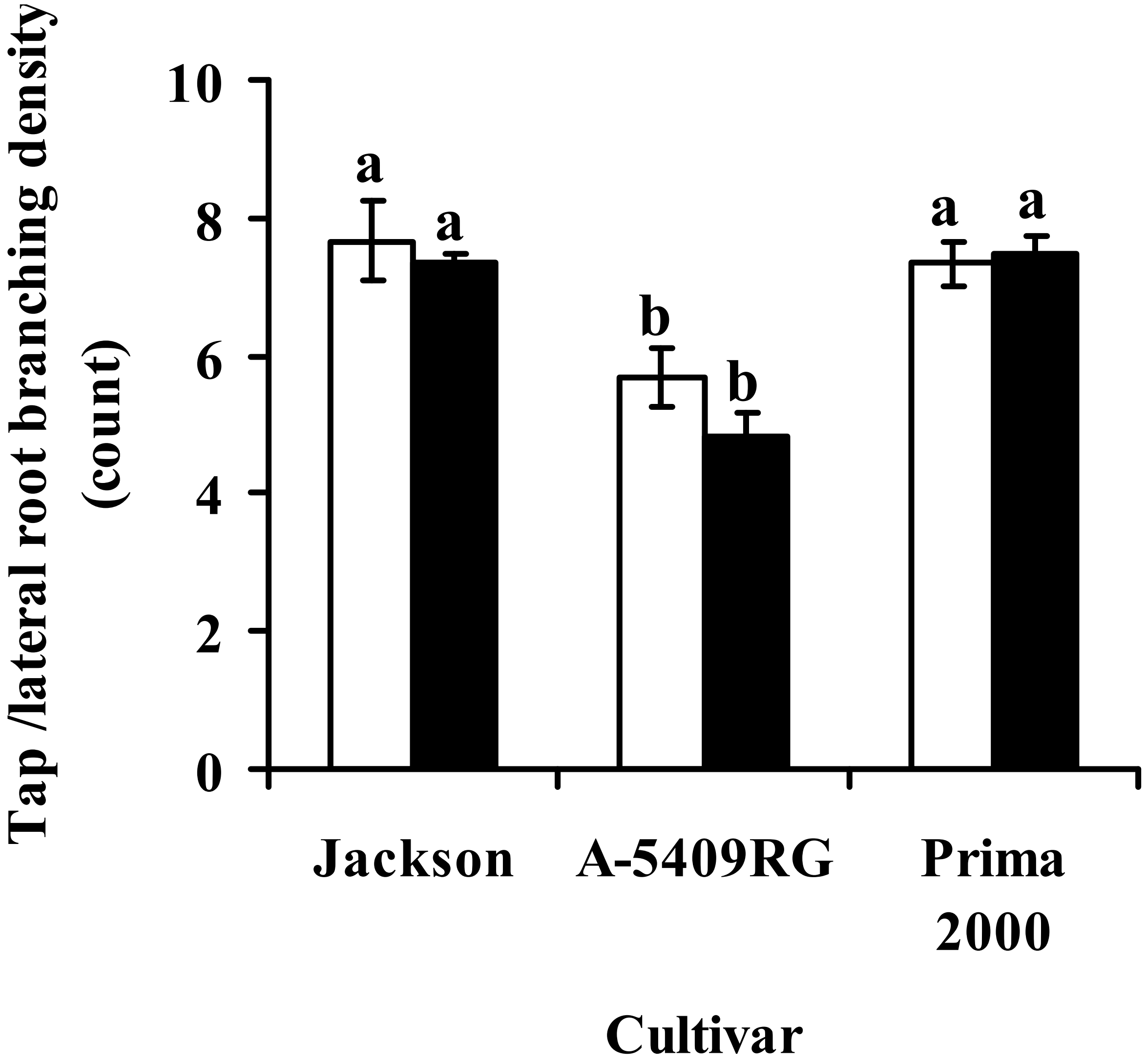


Figure 6.3 The performance of three soybean cultivars for root architecture traits tap root branching (open bars) and lateral root branching (closed bars). Values shown are means ± SEM of three repetitions of three individual plants per plot for each cultivar (nine plants) after one month of drought exposure. The significance letters obtained by the analysis using mean separation LSmeans Student's t-test (P = 0.05).

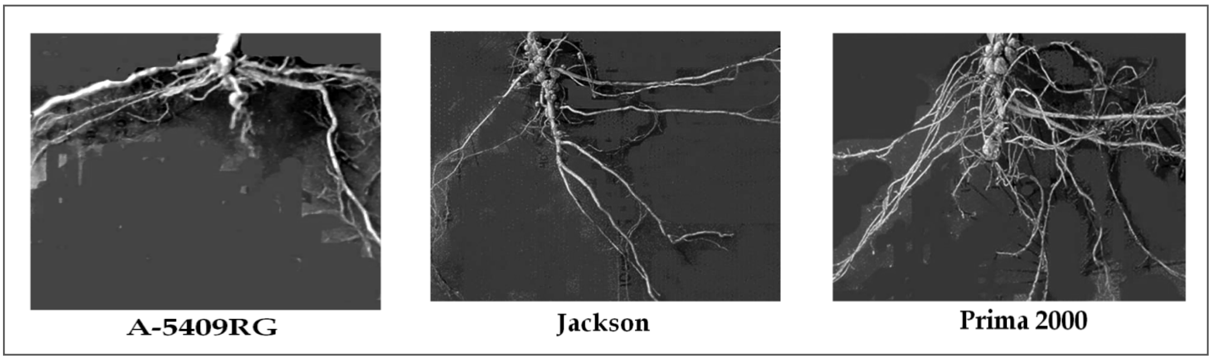

A-5409RG Jackson Prima 2000

Figure 6.4 A comparison of root phenotypes in three soybean cultivars grown under drought. Photos were taken one month after exposure to drought in the field.

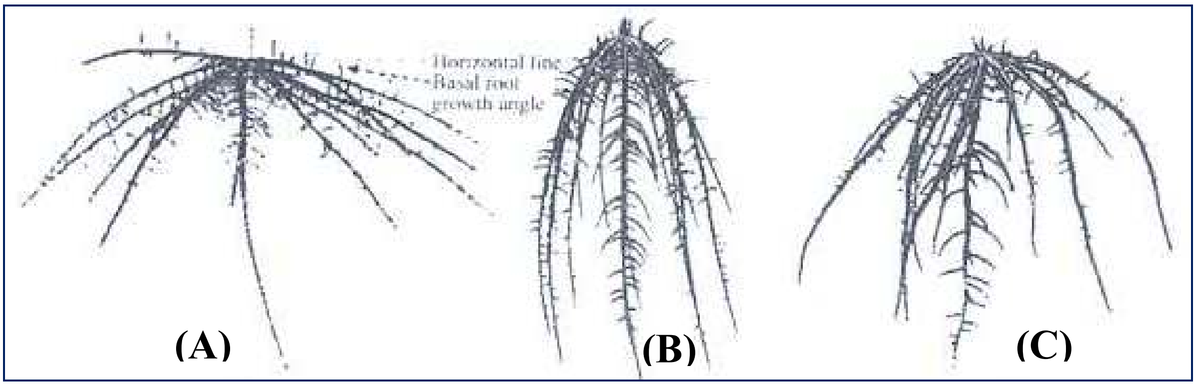


Figure 6.5 Computer simulation of three representative root images representing shallow (A), deep (B) and intermediate (C) roots of soybean obtained in field experiment (Zhao et al., 2004).

6.4.3 Plant biomass, phenology and seed yield

Two way analysis of variance for plant biomass as measured at flowering reveal significant different for cultivar and water level interaction only for leaf dry mass. However, all plant biomasses measured at mid pod filling stage (leaf, pod and total) except stem biomass and seed yield revealed significant difference for cultivar X water level interaction (Appendix 10). For further observation and comparison of the tested cultivars under study, treatment comparison as well was one way ANOVA was conducted. As a result, under drought and well-watered growth conditions, the three cultivars had significantly different ($P<0.05$) leaf, stem and total biomass both at the flowering and mid pod filling stage (including pod mass) (Table 6.3). Under both water regimes, Prima 2000 had significantly higher ($P<0.05$) dry mass (leaf, stem, pod) than the two other cultivars. Drought caused a significant reduction of total biomass at flowering by 21%, 25% and 40% for Prima, Jackson and A5409RG, respectively.

Jackson was the earliest maturing cultivar maturing at 81 days under drought and 90 days under well-watered growth condition. The other two cultivars exhibited similar maturity date of 98-101 days under drought and 115-118 days under well-watered conditions (data not shown).

Under well-watered condition, while the three soybean cultivars revealed non-significant difference for pod harvest index (PHI) (Figure 6.6). Prima 2000 and A5409RG were had higher seed yield (4 to 4.4 t/ha) than Jackson (2.1 t/ha) (Table 6.3). However under drought stress, significant cultivars difference were existed for PHI with Prima 2000 and Jackson exhibited higher Pod harvest index than A5409RG (Figure 6.6), nevertheless, Prima 2000 were had a significantly higher ($P<0.05$) seed yield (2.4 t/ha) than the two other cultivars, Jackson (1.7 tones/ha) and A5409RG (1.9 t/ha) (Table 6.3). Further, when seed yield accumulation per day was calculated under well-watered condition,, Jackson revealed lower but under drought condition both Jackson (21 kg/ha/day) and Prima 2000 (24 kg/ha/day) exhibited higher seed yield accumulation per day than A5409RG (19 kg/ha/day) (Table 6.4). Leaf biomass accumulation per day between flowering and mid-pod filling stage under drought condition were higher for Jackson and Prima 2000, nevertheless, under well-watered condition the three cultivars exhibited similar performance. However, regardless of the water regime, stem biomass accumulation per day was similar for the tested cultivars (Table 6.4).

Table 6.3 Performance of three soybean cultivars for biomass at flowering (Fl) and mid pod filling stage (MPF) and seed yield under well-watered and drought conditions.

Cultivar	**Leaf dry mass at Fl(g)**	**Stem dry mass at Fl(g)**	**Total biomass Fl (g)**	**Leaf dry mass at MPF(g)**	**Stem dry mass at MPF(g)**	**Pod dry mass MPF(g)**	**Total biomass MPF(g)**	**Seed yield (kg/ha)**
<u>Well-watered</u>								
Jackson	5.88±0.13b	5.08±0.27b	10.97±0.37c	22.86±0.32b	25.1317±0.32b	10.40±0.29b	58.39±0.63b	2076.07±117.0b
A5409RG	6.59±0.30ab	5.78±0.24ab	12.37±0.22b	28.66±0.45a	28.9917±0.44a	13.29±0.48a	70.94±0.66a	4060.38±83.51a
Prima 2000	7.27±0.23a	6.43±0.16a	13.71±0.32a	29.86±1.22a	28.2050±1.25a	13.87±0.40a	71.93±1.68a	4425.12±123.6a
Significance	**	**	**	**	**	**	**	**
<u>Drought</u>								
Jackson	4.05±0.93b	4.21±0.17b	8.25±0.18b	21.69±0.22b	21.16±0.43b	9.38±0.13b	52.23±0.46b	1709.63±18.28b
A5409RG	3.77±0.13b	3.65±0.07b	7.42±0.15b	23.15±0.49b	24.40±0.51ab	7.85±0.38c	55.40±0.96b	1868.21±28.23b
Prima 2000	5.96±0.26a	4.88±0.20a	10.84±0.34a	26.47±0.63a	26.22±2.13a	11.20±0.35a	63.82±2.57a	2365.04±35.08a
Significance	**	**	**	**	*	**	**	**

Data represent the mean ± SEM of three replications under both well watered and drought conditions. Biomass at flowering and mid pod filling stage was taken on six representative individual plants per plot. Different letter within a column denote a significant difference ($P<0.05$).

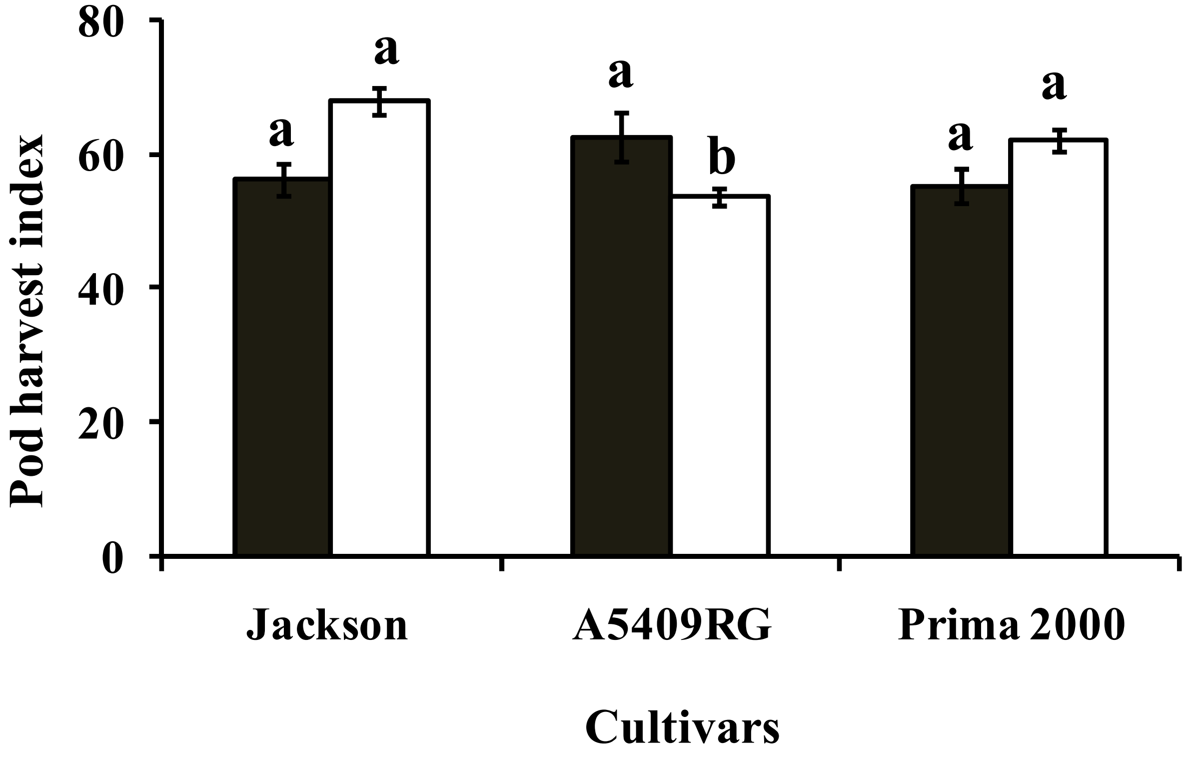


Figure 6.6 Pod harvest index of three soybean cultivar measured at harvest, closed bars indicates for well-watered and open bars for drought stressed treatments. Results are means ± SEM of three repetitions per cultivars. Means with the same letter above the bars are not significantly different as tested by LSmeans Student's t-test (P=0.05).

Table 6.4 Seed yield and biomass accumulation of three soybean cultivars per day. This has been done by dividing seed yield with maturity day, biomass accumulation per day for leaves and stems between days of flowering (Fl) and mid-pod filling (MPF) of the cultivars, by dividing the difference of biomasses with the difference of the days between flowering and maturity.

Cultivars	**Yield (kg/ha)/day**	**Biomass accumulation per day between Fl and MPF stage**	
		Leaf (g/day)	**Stem (g/day)**
Well-watered			
Jackson	25.63	0.33	0.39
A5409RG	40.81	0.32	0.33
Prima 2000	44.47	0.33	0.31
Drought			
Jackson	21.11	0.35	0.33
A5409RG	18.78	0.28	0.30
Prima 2000	23.77	0.30	0.31

6.4.4 Nodule size, carbon isotope discrimination /^{15}N natural abundance

The nodule size of the three soybean cultivars was not significantly different under non-stress conditions (data not shown). However, under drought nodule size differed significantly between cultivars and Jackson and Prima 2000 had larger nodules (4.6 mm) than A-5409RG (3.7 mm) (Figure 6.7).

Analysis for two ways ANOVA for CID determined for both shoot and seed was not significant for the interaction of water treatment and cultivar. As a result for assessing the cultivar performance one way analysis of variance and treatment comparison was conducted. Thus, *c*arbon isotope discrimination under well-watered conditions was significantly different between the tested soybean cultivars and reference non-fixing common bean lines, however, within soybean cultivars there was no significant difference (Table 6.5). Nevertheless, under drought condition, CID was significantly different ($P<0.05$) among the tested soybean cultivars as well as with the reference lines. As a result among the tested soybean cultivars Prima 2000 expressed the lowest CID value followed by Jackson. Regardless of the water regime, the non-fixing reference bean lines had higher CID values than the soybean cultivars (Table 6.5). Nevertheless, seed CID of soybean cultivars was not significantly different under either water regime (data not shown).

Seed δ^{15}N was not significantly different among tested soybean cultivars under both well-watered and drought conditions (data not shown). However, the performance of the tested soybean cultivars and non-nodulating reference beans for δ^{15}N values determined for shoot revealed significant differences under both water regimes. There was no significant difference

(P>0.05) in $\delta^{15}N$ among soybeans under well-watered conditions, but under drought, Jackson and Prima 2000 had lower $\delta^{15}N$ values (-2.28‰ to-2.55‰) than A-5409RG (-1.19‰) or the reference bean lines (1.65‰ to 2.83‰) (Table 6.5). Further, a significant difference (P<0.05) was found between soybean cultivars and reference bean lines for shoot %N under both well-watered and drought conditions. Under well-watered condition, shoot %N ranged from (3.7-4.2%) for soybean and for the reference bean lines between 1.5-1.9%, although there was no significant difference (P>0.005) among the tested soybean cultivars. Under drought, Jackson and Prima 2000 had lower $\delta^{15}N$ values and the highest shoot %N (average 4%). This was significantly different (P<0.05) to A-5409RG (3%) and the reference bean lines (on average 1.6%) (Table 6.5).

The three soybean cultivars had comparable performance under well-watered conditions for percent of nitrogen derived from atmosphere (%Ndfa). However, under drought Prima 2000 and Jackson had significantly higher (P<0.05) (65-70%) %Ndfa than A5409RG (55%) (Table 6.6). Regardless of the water regime, the amount of nitrogen fixed per hectare was higher for Prima 2000 (146 kg/ha with well-watered and 106 kg/ha with drought conditions) than the other two cultivars tested, (117 kg/ha for Jackson and 100 kg/ha for A5409RG) with well-watered and (80 kg/ha, for Jackson and 55 kg/ha, for A5409RG) with drought conditions (Table 6.6)

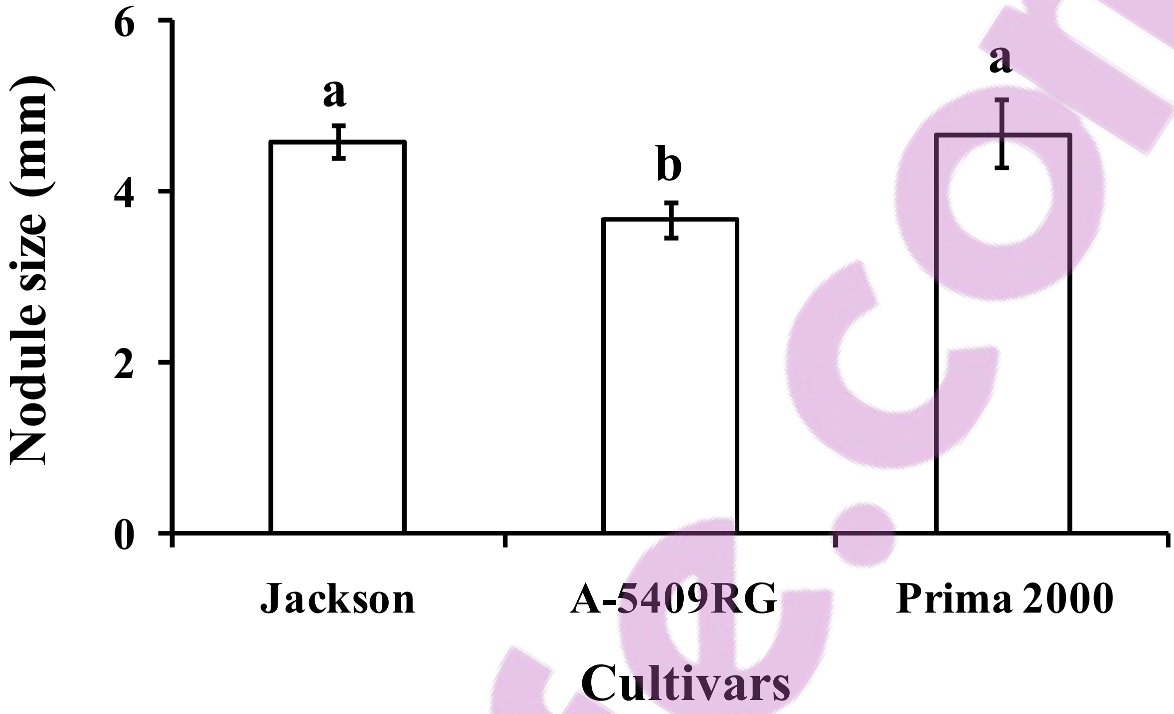


Figure 6.7 Nodules size of three soybean lines grown in the field at drought conditions. Data represent mean ± SEM of four replicates per plot (for twelve individual plants per cultivar) after exposure for one month to drought. Means followed by the same letter on the top of bars are not significantly different (P<0.05).

Table 6.5 Performance after 28 days drought stress of three soybean cultivars and two non-fixing reference common bean lines for the analysis of shoot (CID, $\delta^{15}N$ and %N). Result is the mean of three replicates (each replicate from a composite sample of three plant samples) for each soybean cultivar and reference bean line for every water regime.

Cultivars/ lines	**Shoot CID**		**Shoot $\delta^{15}N$**		**Shoot %N**	
	Well-watered	**Drought**	**Well-watered**	**Drought**	**Well-watered**	**Drought**
N-fixing (soybean)						
Jackson	21.53±0.33b	20.242 ±0.17bc	-2.727±0.42b	-2.280±0.64bc	3.82±0.35a	4.06±0.08a
A-5409RG	21.291±0.11b	20.854 ±0.02ab	-3.112±0.38b	-1.187±0.57b	3.74±0.19a	3.01±0.3b
Prima 2000	21.293±0.2b	19.513±0.41c	-3.322±0.09b	-2.546±0.29c	4.24±0.08a	3.97±0.1a
Non-fixing (bean)						
DOR 364-NN	22.223 ±0.01a	21.337±0.41a	0.066±1.14a	2.834±0.06a	1.78±0.22b	1.88±0.08c
BAT 477-NN	22.293±0.13a	21.677±0.09a	1.735±0.25a	1.650±0.04a	1.45±0.03b	1.34±0.06c
P-value	*0.0059*	*0.0016*	*0.0003*	*<0.0001*	*<0.0001*	*<0.0001*
Significance	**	**	**	**	**	**

Significance level was determined using ANOVA (** P<0.001, and *P<0.05) and difference between treatment means were determined using the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different.

Table 6.6 Percent nitrogen derived from the atmosphere (%Ndfa) and fixed N (g/plot) for shoot samples of three soybean cultivars using LSmeans Student's t-test mean separation (P=0.05). The result is the mean ± SEM of three replicates (each replicate from a composite sample of three plant samples) for each soybean cultivars and reference bean lines every water regime.

Cultivars	**%Ndfa**		**Fixed N (kg/ha)**	
	Well-watered	**Drought**	**Well-watered**	**Drought**
Jackson	85.113±0.34a	64.47±0.88ab	117.11±1.69b	80.39±6.2b
A-5409RG	81.133±0.21a	54.80±0.30b	100.13±1.86b	54.53±3.1c
Prima 2000	73.852±0.66a	69.66±0.50a	146.18±3.2a	105.6±4.9a
P-value	*0.4811*	*0.0371*	*0.016*	*<0.0001*
Significance	*ns*	*	*	**

Significance level was determined using ANOVA (** P<0.001 * P<0.05 and ns P>0.05) and difference between treatment mean was determined by the LSmeans Student's t-test. Means followed by the same letter within the column are not significantly different.

6.4.5 Association between carbon isotope discrimination and ^{15}N natural abundance traits with seed yield and root traits

Carbon isotope discrimination was highly significant ($P<0.01$) and positively correlated with $\delta^{15}N$ and also with SNF efficiency traits (shoot %N and %Ndfa) under both water regimes (Table 6.7). Further, there was a significant association of CID with seed yield under both well–watered ($r = -0.63$, $P<0.05$) and drought ($r = -0.83$, $P<0.01$) conditions (Table 6.7).

The relationship of shoot $\delta^{15}N$ was significantly related with %N under both well-watered ($P<0.01$, $r = -0.94$) and drought ($P <0.05$, $r = -0.85$) conditions. Further, both %N and %Ndfa had a significant and positive relationship with seed yield under well-watered and drought conditions. Accordingly, the association for %N was ($r = 0.8$, $P<0.01$) under both growth conditions and %Ndfa ($r = 0.67$, $P<0.05$) under well-watered and ($r = 0.95$, $P<0.01$) under drought conditions, respectively. Irrespective of the water regime, a highly significant ($P<0.01$) association was found between $\delta^{15}N$ and seed yield ($r = -0.67$, well-watered and $r= -0.91$, drought) (Table 6.7).

Only a weak correlation of $\delta^{15}N$ with both root architecture and morphology traits was found. Moreover, there was no significant association of CID with root traits under well-watered conditions (data not shown). However, under drought CID was significantly positively associated with lateral root diameter ($r = 0.77$) but significantly negatively correlated with tap root diameter ($r = -0.52$). Further, root morphology traits (root length, surface area and volume) had a significant ($P<0.05$) negative association with CID under drought (Table 6.8).

Table 6.7 Association between shoot CID, δ^{15}N, and % N for (three soybean cultivars and two non-fixing reference common bean lines) of three soybean cultivars. Data are from pooled data from the 3 soybean cultivars of three replicates (each replicate from a composite sample of three plant samples) of plants grown under well-watered and drought conditions.

Traits	**Shoot CID**	**Shoot δ^{15}N**	**Shoot %N**	**%Ndfa**
Well-watered				
Shoot %C	-0.690*			
Shoot δ^{15}N	0.760**	1.000		
Shoot %N	-0.835**	-0.935**	1.000	
%Ndfa	-0.760*	-1.000**	0.935*	1.000
Seed yield	-0.631*	-0.666**	0.805**	0.666*
Drought				
Shoot %C	-0.521*			
Shoot δ^{15}N	0.821**	1.000		
Shoot %N	-0.795**	-0.849**	1.000	
%Ndfa	-0.917**	-0.717*	0.417ns	1.000
Seed yield	-0.832**	-0.905**	0.838**	0.948**

Correlation is highly significant (** P<0.001), significant (*P<0.05) and non significant (ns P>0.05)

Table 6.8 Correlation between shoot carbon isotope discrimination and root morphology or architectural traits of three soybean cultivars. Data obtained from pooled data of 3 cultivars and from three replicates (each replicate from a composite sample of three plant samples) of plants grown under drought.

Trait	**Trait**	**Correlation coefficient (r)**	**P-value**
	Root architecture		
Carbon isotope discrimination	Lateral diameter	0.691	0.0159*
	Lateral branching	-0.172	0.7759ns
	Tap diameter	-0.536	0.0445*
	Tap branching	0.101	0.7963ns
	Root morphology		
	Length	-0.476	0.0457*
	Surface area	-0.607	0.0324*
	Volume	-0.712	0.0444*

*indicates correlation is significantly different ($P<0.05$), ns indicates correlation is non-significant ($P>0.05$).

6.5 Discussion

In this study drought had a significant effect on both root morphology (Table 6.1) and architectural parameters (Table 6.2, Figure 6.3 and 6.4). As roots are the primary sensor of water deficit, the ability of the plant to adapt to an altered soil environment depends on root developmental plasticity attributes (morphology and architecture) (Lynch et al., 2005). As a result, there was a significant cultivar difference in the response of root traits under drought in this study and the tested cultivars can be classified into three types. A-5409RG had a shallower root system with a less than 40^0 basal/lateral root angle (Figure 6.4) classified as type A (Figure 6.5) (Zhao et al., 2004). Further, plants of this cultivar had a short and slender tap root (Figure 6.4), thicker and few lateral (basal) roots, a lower branching density (Figures 6.3 and 6.4, Table 6.2), smaller root length, surface area and volume (Table 6.1). In contrast, Jackson had a deeper root system with a basal/lateral root angle greater than 60^0, (Figure 6.4) classified as type B (Figure 6.5) (Zhao et al., 2004). This cultivar had a long and thicker tap root system, many basal roots (Figure 6.4 and Table 6.2) and therefore a high branching density (Figure 6.3) as well as a higher root length, surface area and volume (Table 6.1). Cultivar Prima 2000 had an intermediate root system with a basal root angle between $40\text{-}60^0$ (Figure 6.4), classified as type C (Figure 6.5) (Zhao et al., 2004), with a long and deep tap root, numerous basal roots and consequently higher branching density (Figures 6.3 and 6.4), and also higher root length, surface area and volume (Table 6.1).

The two cultivars, Jackson and Prima 2000, were further able to extend their root system to adapt to drought (Tables 6.1 and 6.2, Figures 6.3 and 6.4). However under drought condition, Prima had a significantly higher yield (2.4 t/ha) than Jackson (1.7 t/ha). But estimates of yield per day and leaf biomass accumulation per day between flowering and mid pod filling stage under drought indicates that Jackson and Prima 2000 had greater ability to accumulate photosynthates and the higher values of pod harvest index indicate that these two cultivars also have greater ability to mobilize photosynthates to seed compared to A5409RG. Hence, these shows, plant traits that can help to evaluate the proportion of photosynthates that are partitioned to seed yield might contribute to the selection of more efficient genotypes. Cultivars Prima 2000 and Jackson showed better performance under drought suggesting that biomass accumulation and partitioning towards the developing seeds are key physiological factors in the adaptation to drought. Therefore, although the cultivar with a longer maturation produces more yield than with a short maturation, the rate of daily seed yield accumulation (rate of partitioning) is indispensable for comparing adaptation under drought. Accordingly, these two cultivars could serve as parents for future improvement of seed yield under drought stress. Nevertheless, the low yield of Jackson relative to Prima 2000 was due to the duration to attain maturity for Jackson which was short to convert assimilated carbohydrate and absorbed nutrients to grain yield.

The amount of plant biomass and grain yield depends on the amount of photosynthetically active radiation (PAR) interception by the plant canopy and partitioning of the photosynthetic product to harvestable form (Mayers et al., 1991). This has a direct relationship with the duration of the crop growth (days to attain maturity and relative duration of pod filling). These observations might suggest that productivity under long-term drought is related to phonology in soybean (Lawn and James, 2011). In general, Prima 2000 had a better root architecture (tap and lateral root branching) and morphology traits (root length, surface area, and volume) as well as plant shoot biomass under both water regimes. It appears that there is a good balance between root and shoot growth in this cultivar. Jackson, regardless of the water regime, had shorter shoots and a deeper root system which might contribute to water saving mechanisms during drought stress. Results obtained from this study are in agreement with the observations made by Zhao et al. (2004) from a soybean core collection study that highlighted the need to match root and shoot architectures for efficiently converting assimilated carbon as well as absorbed nutrients and water to harvestable grain yield and for improving drought-tolerance in soybean.

In this study, soybean cultivars Jackson and Prima 2000 with better root performance had also low shoot CID values. Condon et al. (1990); Farquhar and Richards (1984) previously reported that CID is negatively associated with water use efficiency under drought. Lower CID has been considered as a marker for better water use efficiency (Farquhar et al., 1989). Lower CID could be either due to lower stomatal conductance which has been found in rice (Dingkuhn et al., 1989) or greater CO_2 assimilation found in common bean (Ehleringer, 1990) and winter wheat (Morgan et al., 1993). The better performing soybean cultivar Prima had an enhanced root system (root morphology and architecture) under drought in comparison to the susceptible cultivar (A5409RG). This would allow better opening of the stomata and improved CO_2 assimilation. One important factor affecting the guard cell turgor is the leaf water status. Plants with a deep and dense root system (enhanced root system flexibility) under drought helps for better water extraction from the soil allowing capturing more water keeping both shoot turgidity and opening of stomata. As a result, the plant will assimilate more CO_2.

The relatively higher amount of chlorophyll found for Prima 2000 also supports the view that this cultivar is less affected by drought. These characteristics were also found for Prima 2000 in the phytotron studies with better CO_2 assimilation, stomatal conductance and WUE (Fenta et al., 2011). More importantly, the negative association of CID with root morphology traits (length, area, and volume) (Table 6.8) as well as with seed yield under drought (Table 6.7) indicates the importance of maintaining the plant water status through utilization of available water for continuous CO_2 assimilation under water-limited growth conditions. Therefore, these observations suggests, this study support the view that low CID (high WUE) could be due to enhanced photosynthetic assimilation per unit water transpired (transpiration efficiency).

Carbon isotope discrimination was also negatively associated with SNF efficiency (shoot %N and %Ndfa) and positively associated with $\delta^{15}N$, which is a desirable association, as the value of $\delta^{15}N$ is negative. This further ascertains the close intimacy of carbon and nitrogen fixation. Previous findings in beans have also indicated that nitrogen accessibility to the plant increases concurrently with increased WUE (Caemmerer and Farquhar 1984). Farquhar and Richards (1984) have also highlighted the importance of better nitrogen availability for enhanced CO_2 assimilation. In this study, Prima 2000 had better root traits, biomass and productivity as well as higher SNF ability (146 kg/ha, under drought) (Table 6.6) and shoot %N (Table 6.5) in comparison to the other cultivars tested that performed less for the measured traits. This shows that accessibility of nitrogen (absorption from the soil and N fixation from the air) is highly affected by water availability.

Since water is absorbed by the plant mostly through mass flow, which is also highly affected by contact of the root surface area to the soil, the response of the root system under drought is fundamental for drought tolerance and productivity under drought. The water supply to nodules is via the phylum (Walsh et al., 1989). Since drought affects the volumetric flow in the phylum, the relative water content of nodules decreases (Purcell and Sinclair, 1995) reducing the nodule size. Maintaining the water supply of the phylum improves the water status of nodules and facilitates the solute flow from the nodule (Purcell and Sinclair, 1995). This ultimately helps for improved SNF of the plant. Therefore, effect of drought stress on nodule size would be one of the important traits which should be considered during drought screening.

According to multivariate analysis of variance for cultivar and water treatment soybean cultivars exhibited consistent performance for plant performance traits for CID and SNF traits under the two water treatment however, plant biomasses measured at mid pod filling stage (leaf, pod and total) as well as leaf biomass at flowering, and for root morphology traits water stress act as a moderator on affecting the response of soybean cultivars for these traits. These traits especially, biomass and seed yield was found to be noticeable traits for selecting specific cultivar adaptable for specific water regime. As a result, both Prima 2000 and A5409RG can be selected for well-watered condition but, under drought stress Prima 2000 (Table 6.3).

Overall, field experiments confirmed the previous phytotron results of superior performance of Prima 2000 under drought. The study also verified the importance of root system architecture and morphology for providing drought tolerance in soybean. Results further suggest that root architectural traits of tap and lateral roots (thickness and branching density) and morphological traits (root length, surface area and volume) could be used as simple and quick performance evaluation tools in future soybean improvement programs. Also, for the first time, the strong association of CID (WUE) with $\delta^{15}N$ (SNF), root traits as well as seed yield in soybean exposed to drought has been ascertained. This also demonstrates the importance of CID (WUE) as a potent selection criterion for enhanced soybean performance under drought. In addition, research findings suggest that higher performance in CID under drought stress may be due to higher CO_2 assimilation and better N_2 fixation resulting in better root system architecture and morphology of the drought tolerant cultivar Prima 2000 for maintain the water status of the plant for efficient biological activity.

CHAPTER SEVEN

GENERAL DISCUSSION AND FUTURE RESEARCH

The overall aim of this PhD study was to investigate performance of different bean and soybean cultivars under drought by using selected morphological or physiological phenotypic markers (traits), and to investigate the potential of these performance markers under environmentally controlled and/or field growth conditions. In general, results obtained in field experiments were in agreement with the findings in environmentally controlled phytotron experiments. In particular, in this study support has been found for the hypothesis that common bean and soybean have a similar morphological or physiological phenotypic basis of drought adaptation permitting the use of identical performance markers for selection of more drought-tolerant cultivars under both types of growth environments.

A novel finding of this study was that of associating better performance of both legumes under drought with both developmental plasticity of the root system and with enhanced photosynthetic carbon assimilation: WUE (water use efficiency), SNF (symbiotic nitrogen fixation) and plant biomass production. Results from field experiments clearly demonstrated that particular root morphology and architectural traits are important for better performance under drought and that these root traits are associated with better seed yield. These field experiments further allowed determining the contribution of SNF to %N, and elucidating the relationship of SNF with WUE. Specifically the root architectural traits (1^{st} whorl angle, basal root number and adventitious root branching density) and root morphological traits (root length, area and volume) were significantly associated with better seed yield under drought. These root traits might be important selection criteria in future bean improvement program to select for drought tolerance.

A further new finding was the direct relationship between both carbon isotope discrimination (i.e., WUE) and SNF, and root morphological (root length, area and volume) as well as architectural (basal root number, 1st as well as 2nd whorl angles) traits in common bean under drought. The capacity to maintain nodule size in common bean under water-limited condition was also associated with these traits. Also, the direct relationship found between CID (WUE) and SNF with PHI and seed yield under drought stress condition has further ascertained the importance of maintaining the water status of the plant through enhanced plasticity of root architecture for better performance under drought as well as for higher SNF ability.

For soybean, the importance of the root morphology (root length, surface area and volume) for drought tolerance was also clearly shown. In addition, the study provided new knowledge about an existing strong association of CID (WUE) with $\delta^{15}N$ (SNF), root traits and seed yield in soybean under drought. More importantly, this research revealed, for the first time, that higher WUE (lower CID value) is related to higher photosynthetic assimilation and better N_2 fixation as well as improved root system architecture and morphology that contribute to maintaining the water status of the plant for efficient biological activity under drought stress.

A further novel aspect of the study was the finding that the ability to sustain shoot biomass under nitrogen limited conditions is important for selecting improved drought tolerant legume germplasm (soybean and common bean in this study). By using correlation and principal component analysis, it was shown in this study that maintaining SNF ability under controlled conditions of drought stress in legumes is strongly associated with their ability for improved CO_2 assimilation and stomatal conductance. Also the importance of the leaf water status and IWUE

(CO_2 assimilation/stomatal conductance) for better performance under drought was verified. Cultivars, such as Prima 2000 (soybean), BAT 477 and BT_34-1-1 (common bean), have the capacity for maintaining IWUE for a longer period which is essential for maintaining better SNF ability for a longer time that affects plant development. Therefore, enhanced CO_2 assimilation through better stomatal conductance together with enhanced root and shoot development are important plant processes for drought-tolerant soybean and common bean for maintaining the balance between the shoot and the root biomass partitioning.

Generally, this study has generated new knowledge about the use of physiological markers (traits) that can be used for legume evaluation under drought suitable for both phytotron and field studies. This includes shoot biomass, WUE (IWUE/CID), nodule size/mass, and SNF (ARA/$\delta^{15}N$). Markers highly appropriate for phytotron studies include determination of biomass, leaf area, gas exchange parameters and leaf water potential. For field evaluation root architectural and morphological traits were found to be important markers that can be used in a legume improvement program. Particularly in common bean measurement of whorl angle, number of basal roots and number as well as branching density of adventitious roots would also be an important addition. Figure 8.1 presents a proposed overall inter-relationship of the performance markers based on the outcome of this study. Finally, a further outcome of this study would be suggesting to modify the previously proposed function of grain yield set by Passioura (1996) with [Y= EUW x WUE x HI] into Y= EUW x WUE x HI x SNF, for legumes with Y representing grain yield, EUW the effective use of water through enhanced root development and HI the harvest index where PHI (the pod harvest index) is an important sub component of HI..

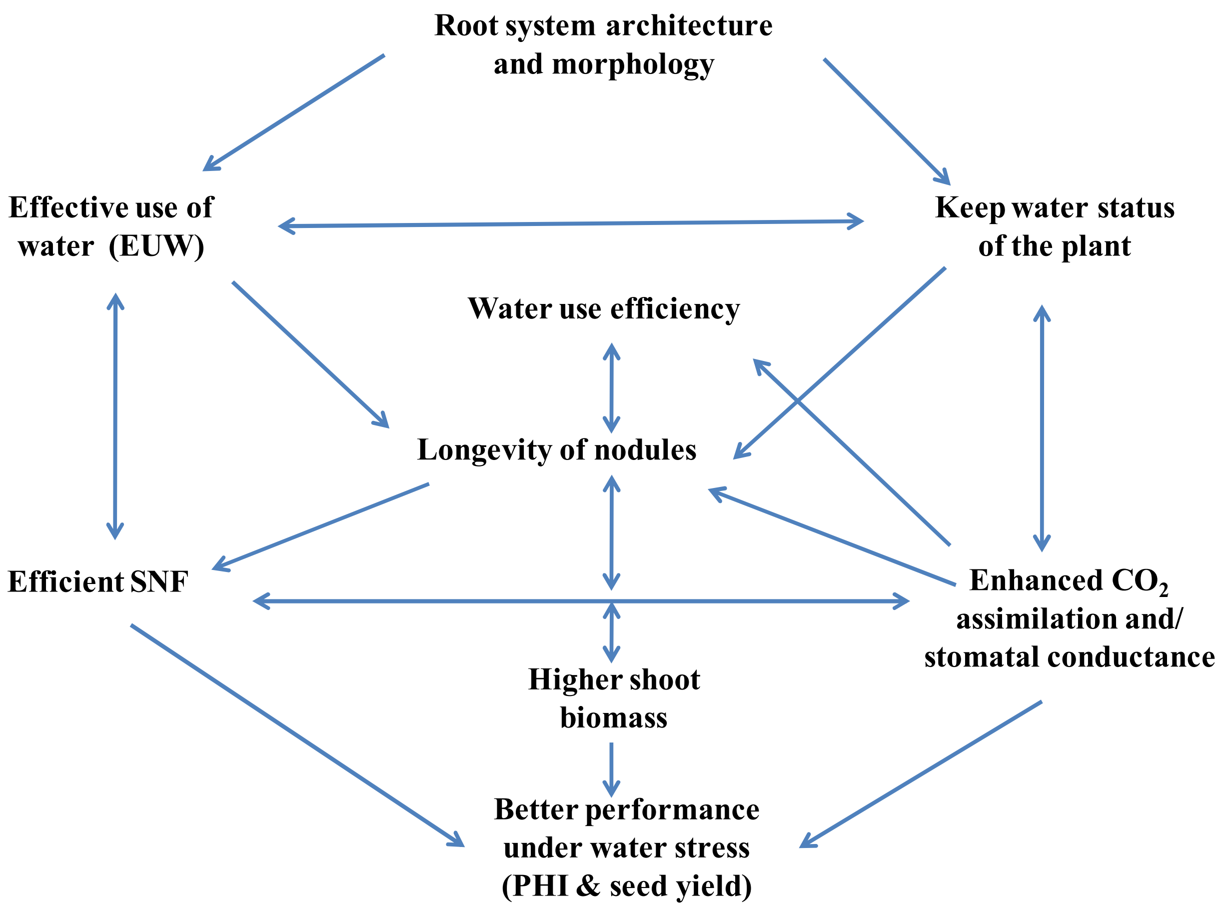


Figure 7.1 Proposed schematic representation inter-relationship of the physiological and morphological performance markers under drought based on results obtained in this study.

Future research actions should focus first on implementing identified markers (traits) into the existing bean program in Ethiopia. The applicability of these markers in a large germplasm screening program should be evaluated and in particular root performance markers, which have not been used in varietal improvement programs, and these should be evaluated for their potential to be easily applicable in such large programs. A second future action should include carrying out crossings with commercial cultivars and studying the heritability of these markers. Wider application to tropical legumes, such as chickpea, cowpea and mungbean, which are of relevance for Ethiopia, should be a third future research action. Finally, a fourth future research action should be the application of these markers for providing superior performance under various other abiotic stresses of relevance to Ethiopia such as low P, aluminium toxic acid soils, and low fertility soils. The root architectural growth is highly affected by the fertility of the soil, especially low P, which is a serious problem for crop production for over 70% of agricultural land throughout the world. Therefore, for identification of legumes for multiple stresses or for better soil P acquisition and for drought would be a major challenge for breeders. Therefore, the need to understand and investigate morpho-physiological plant processes or traits, particularly root system traits responsible for multiple stresses, would be a vital addition to bean breeding programs.

REFERENCES

Abate, T. 2012. Four Seasons of Learning and Engaging Smallholder Farmers Progress of Phase International Crops Research Institute for the Semi-Arid Tropics. Nairobi, Kenya.

Acosta-Gallegos, J., and J.W. White. 1994. Phenological Plasticity as an Adaptation by Common Bean to Rainfed Environments. Crop Science 35:199-204.

Acosta Gallegos, J.A. 1988. Selection of common bean (*Phaseolus vulgaris* L.) genotypes with enhanced drought tolerance and biological nitrogen fixation, Ph.D.Thesis Michigan State University, East Lansing, Michigan.

ÅGren, A.I., and O. Franklin. 2003. Root :Shoot Ratios, Optimization and Nitrogen Productivity. Annals of Botany 92:795-800.

ÅGren, G.I., and T. Ingestad. 1987. Root: Shoot ratio as a balance between nitrogen productivity and photosynthesis. Plant, Cell & Environment 10:579-586.

Ainsworth, E.A., C.R. Yendrek, J.A. Skoneczka, and S.P. Long. 2011. Accelerating yield potential in soybean: potential targets for biotechnological improvement. Plant, Cell & Environment 35:38-52.

Alsina, M.M., D.R. Smart, T. Bauerle, F. de Herralde, C. Biel, C. Stockert, C. Negron, and R. Save. 2011. Seasonal changes of whole root system conductance by a drought-tolerant grape root system. Journal of Experimental Botany 62:99-109.

Amede, T., and S. Schubert. 2003. Mechanisms of drought resistance in grain legumes I: Osmotic adjustment. SINET: Ethiopian Journal of Science 26:37-46.

Anjum, S.A., L. Wang, M. Farooq, I. Khan, and L. Xue. 2011. Methyl Jasmonate-Induced Alteration in Lipid Peroxidation, Antioxidative Defence System and Yield in Soybean Under Drought. Journal of Agronomy and Crop Science 197:296-301.

Anyia, A.O., D.J.Archambault, J.J. Slaski, and J.M.Nyachiro. 2005. Carbon isotope discrimination as a selection criterion for improved water use efficiency and productivity of barley on the prairies. 18thNorth American Barley Researchers Workshop and 4thCanadian Barley Symposium. Red Deer, Alberta, Canada:118-123.

Ao, J., J. Fu, J.Tian, X.Yan, and H. Liao. 2010. Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. Functional Plant Biology 37:304-312.

Araus, J.L., C. Sánchez, and G.O. Edmeades. 2011. Phenotyping maize for adaptation to drought. Monneveux P. and Ribaut J.M. (eds), Drought phenotyping in crops: from theory to practice. Generation Challenge Programme,pp.259-282.

Araus, J.L., T. Amaro, Casades, J. s, A. Asbati, and M.M. Nachit. 1998. Relationships between ash content, carbon isotope discrimination and yield in durum wheat. Functional Plant Biology 25:835-842.

Arrese-Igor, C., E.M. González, D. Marino, R. Ladrera, E. Larrainzar, and E. Gil-Quintana. 2011. Physiological response of legumes nodules to drought. Plant Stress 5:24-31.

Bacon, M.A. 2004. Water use efficiency in plant biology Blackwell Publishing Oxford, UK.

Baker, N.R. 1996. Photosynthesis and the Environment Springer.

Banziger, M., G. Edmeades, D. Beck, and M. Bellon. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice. Mexico DF CIMMYT.

Bao, F., J. Shen, S.R. Brady, G.K. Muday, T. Asami, and Z. Yang. 2004. Brassinosteroids Interact with Auxin to Promote Lateral Root Development in Arabidopsis. Plant Physiology 134:1624-1631.

Beebe, S.E., IM Rao , Blair MW, and JA Acosta-Gallegos. 2010. Phenotyping common beans for adaptation to drought. In: Drought phenotyping: Theory and practice. (Monneveux P and Ribaut JM, eds.) pp. 311–334. Generation Challenge Programme, Texcoco, Mexico

Beebe, S.E., I. Ochoa, P. Skroch, J. Nienhuis, and J. Tivang. 1995. Genetic Diversity among Common Bean Breeding Lines Developed for Central America. Crop Science 35:1178-1183.

Beebe, S.E., M. Rojas-Pierce, X. Yan, M.W. Blair, F. Pedraza, F. Muñoz, J. Tohme, and J.P. Lynch. 2006. Quantitative Trait Loci for Root Architecture Traits Correlated with Phosphorus Acquisition in Common Bean. Crop Science 46:413-423.

Bergersen, F.J., and G.L. Turner. 1983. An evaluation of ^{15}N methods for estimating nitrogen fixation in a subterranean clover-perennial ryegrass sward. Australian Journal of Agricultural Research 34:391-401.

Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential——are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56:1159-1168.

Blum, A. 2011. Drought resistance - is it really a complex trait? Functional Plant Biology 38:753-757.

Boyer, J.S. 1982. Plant Productivity and Environment. Science 218:443-448.

Boyer, J.S., S.C. Wong, and G.D. Farquhar. 1997. CO_2 and Water Vapor Exchange across Leaf Cuticle (Epidermis) at Various Water Potentials. Plant Physiology 114:185-191.

Brady, N.C., and R.R. Weil. 2008. The nature and properties of soils Prentice Hall.

Brouwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth. J. Agric. Sci. 10:399-408.

Brugnoli, E., and G.D. Farquhar. 2000. Photosynthetic fractionation of carbon isotopes, p. 399-434. In: R.C. Leegood, T.D.Sharkey, and S. von Caemmerer (eds.) Photosynthesis: physiology and metabolism. Advances in photosynthesis, Vol. 9. Clair Academic Publ., The Netherlands.

Buckley, T.N., G.D. Farquhar, and K.A. Mott. 1997. Qualitative effects of patchy stomatal conductance distribution features on gas-exchange calculations. Plant, Cell & Environment 20:867-880.

Casimiro, I., A. Marchant, R.P. Bhalerao, T. Beeckman, S. Dhooge, R. Swarup, N. Graham, D. Inzé, G. Sandberg, P.J. Casero, and M. Bennett. 2001. Auxin Transport Promotes Arabidopsis Lateral Root Initiation. The Plant Cell Online 13:843-852.

Castellanos, J., J.J. Peña-Cabriales, and J.A. Acosta-Gallegos. 1996a. ^{15}N-determined dinitrogen fixation capacity of common bean (*Phaseolus vulgaris*) cultivars under water stress. The Journal of Agricultural Science 126:327–333.

Castellanos, J.Z., J.J. Peña-Cabriales, and J.A. Acosta-Gallegos. 1996b. ^{15}N-determined dinitrogen fixation capacity of common bean (*Phaseolus vulgaris*) cultivars under water stress. The Journal of Agricultural Science 126:327-333.

Cha-um, S., and C. Kirdmanee. 2008. Effect of osmotic stress on proline accumulation, photosynthetic abilities and growth of sugarcane plantlets (*Saccharum officinarum* L.). Pakistan Journal of Botany 40:2541-2552.

Chaves, M.M. 1991. Effects of Water Deficits on Carbon Assimilation. Journal of Experimental Botany 42:1-16.

Chaves, M.M., and M.M. Oliveira. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of Experimental Botany 55:2365-2384.

Chaves, M.M., J.P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought --; from genes to the whole plant. Functional Plant Biology 30:239-264.

Chaves, M.M., J.S. Pereira, J. Maroco, M.L. Rodrigues, C.P.P. Ricardo, M.L. Osorio, I. Carvalho, T. Faria, and C. Pinheiro. 2002. How Plants Cope with Water Stress in the Field? Photosynthesis and Growth. Annals of Botany 89:907-916.

Chen, P., C.H. Sneller, L.C. Purcell, T.R. Sinclair, C.A. King, and T. Ishibashi. 2007. Registration of Soybean Germplasm Lines R01-416F and R01-581F for Improved Yield and Nitrogen Fixation under Drought Stress. Journal of Plant Registration 1:166-167.

CIAT. 1996. Bean program annual report, CIAT, Cali, Colombia.

CIAT. 2007. Bean program annual report, CIAT, Cali, Colombia.

CIAT. 2010. http://www.ciat.cgiar.org/Paginas/index.aspx [Online].

Comstock, J., and J. Ehleringer. 1993. Stomata1 Response to Humidity in Common Bean (*Phaseolus vulgaris*):Implications for Maximum Transpiration Rate, Water-use Efficiency and Productivity. Australian Journal Plant Physiology 20:669-691.

Condon, A.G., G.D. Farquhar, and R.A. Richards. 1990. Genotypic Variation in Carbon Isotope Discrimination and Transpiration Efficiency in Wheat. Leaf Gas Exchange and Whole Plant Studies. Functional Plant Biology 17:9-22.

Condon, A.G., R.A. Richards, G.J. Rebetzke, and G.D. Farquhar. 2002. Improving Intrinsic Water-Use Efficiency and Crop Yield. Crop Science 42:122-131.

Connor, D.J., R.S. Loomis, and K.G. Cassman. 1992. Crop Ecology: Productivity and Management in Agricultural Systems Cambridge University Press.

Coque, M., A. Martin, J. Veyrieras, B. Hirel, and A. Gallais. 2008. Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences. TAG Theoretical and Applied Genetics 117:729-747.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture - not by affecting ATP synthesis. Trends in Plant Science 5:187-188.

Costa Franca, M.G., A.T. Pham Thi, C. Pimentel, R.O. Pereyra Rossiello, Y. Zuily-Fodil, and D. Laffray. 2000. Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environmental and Experimental Botany 43:227-237.

Craufurd, P.Q., R.B. Austin, E. Acevedo, and M.A. Hall. 1991. Carbon isotope discrimination and grain-yield in barley. Field Crops Research 27:301-313.

CSA. 2010. Central Statistics Authority of Ethiopia, Annual Report.

DaCosta, M., and B. Huang. 2006. Changes in Carbon Partitioning and Accumulation Patterns during Drought and Recovery for Colonial Bentgrass, Creeping Bentgrass, and Velvet Bentgrass. Journal of the American Society for Horticultural Science 131:484-490.

Dakora, F.D., and S.O. Keya. 1997. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. Soil Biology and Biochemistry 29:809-817.

De Dorlodot, S., B. Forster, L. Pagès, A. Price, R. Tuberosa, and X. Draye. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends in Plant Science 12:474-481.

De Smet, I., T. Tetsumura, B. De Rybel, N. Frei dit Frey, L. Laplaze, I. Casimiro, R. Swarup, M. Naudts, S. Vanneste, D. Audenaert, D. Inzé, M.J. Bennett, and T. Beeckman. 2007. Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. Development 134:681-690.

Deleens, E., J.B. Cliquet, and J.L. Prioul. 1994. Use of ^{13}C and ^{15}N Plant Label Near Natural Abundance for Monitoring Carbon and Nitrogen Partitioning. Functional Plant Biology 21:133-146.

Denison, R.F. 1998. Decreased oxygen permeability: a universal stress response in legume root nodules. Botanica acta 111:191-192.

Denison, R.F., and B.L. Harter. 1995. Nitrate Effects on Nodule Oxygen Permeability and Leghemoglobin (Nodule Oximetry and Computer Modeling). Plant Physiology 107:1355-1364.

Ding, L., K.J. Wang, G.M. Jiang, D.K. Biswas, H. Xu, L.F. Li, and Y.H. Li. 2005. Effects of Nitrogen Deficiency on Photosynthetic Traits of Maize Hybrids Released in Different Years. Annals of Botany 96:925-930.

Dingkuhn, M., D.S.K. De, K. Dorffling, C. Javellana, and S. Datta. 1989. Varietal differences in leaf water potential, leaf net CO_2 assimilation, conductivity and water use efficiency in upland rice. Australian Journal of Agricultural Research 40:1183-1192.

Djekoun, A., and C. Planchon. 1990. Water Status Effect on Dinitrogen Fixation and Photosynthesis in Soybean. Agronomy Journal 83:316-322.

Ehleringer, J.R. 1988. Correlations between carbon isotope ratio, water use efficiency and yield. p165-191. *In*: J.W. White et al.(ed.) Research in drought tolerant in common bean. CIAT,Cali,Colombia.

Ehleringer, J.R. 1990. Correlations between Carbon Isotope Discrimination and Leaf Conductance to Water Vapor in Common Beans. Plant Physiology 93:1422-1425.

Elazab, A., G. Molero, M.D. Serret, and J.L. Araus. 2012. Root traits and $d^{13}C$ and $d^{18}O$ of durum wheat under different water regimes. Functional Plant Biology 39:379-393.

Esau, K. 1965. Plant anatomy. second ed. Wiley, Michigan, USA.

Evans, J.R. 1983. Nitrogen and Photosynthesis in the Flag Leaf of Wheat (*Triticum aestivum* L.). Plant Physiology 72:297-302.

Evans, J.R., T.D. Sharkey, J.A. Berry, and G.D. Farquhar. 1986. Carbon Isotope Discrimination measured Concurrently with Gas Exchange to Investigate CO_2 Diffusion in Leaves of Higher Plants. Functional Plant Biology 13:281-292.

FAO. 2001. Perfiles nutricionales por paises.Nicaragua. ftp:fao.org/es/esn/nutrition/ncp/nic/pdf. *In*:Beebe, S.E., IM Rao , Blair MW, and JA Acosta-Gallegos. 2010. Phenotyping common beans for adaptation to drought. In: Drought phenotyping: Theory and practice. (Monneveux P and Ribaut JM, eds.) pp. 311–334. Generation Challenge Programme, Texcoco, Mexico

FAO. 2003. http//apps.fao.org/page/collections.

FAO. 2006. FAOSTAT: FAO Stastical Databases. http://faostat.fao.org/.

FAO. 2008. *In*:Alene, A., O. Coulibaly, H. Tefera, S. Boahen, and D. Chikoye. 2011. Targeting Cowpea & Soybean Breeding & Seed Delivery: IITA, 3rd ESA Annual Review & Planning Meeting ;22-26 August 2011, Lilongwe, Malawi.

FAOSTAT. 2010. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor [Online].

Farquhar, G.D., and R.A. Richards. 1984. Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. Functional Plant Biology 11:539-552.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Functional Plant Biology 9:121-137.

Farquhar, G.D., J.R. Ehleringer, and K.T. Hubick. 1989. Carbon Isotope Discrimination and Photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40:503-537.

Fenta, B.A., S.P. Driscoll, K.J. Kunert, and C.H. Foyer. 2011. Characterization of Drought-Tolerance Traits in Nodulated Soya Beans: The Importance of Maintaining Photosynthesis and Shoot Biomass Under Drought-Induced Limitations on Nitrogen Metabolism. Journal of Agronomy and Crop Science 198:92-103.

Fernandez-Luquen˜F., Dendooven L., Munive A. , Corlay-Chee L. , Serrano-Covarrubias L.M. , and Espinosa-Victoria D. 2008. Micro-morphology of common bean (*Phaseolus vulgaris* L.) nodules undergoing senescence. Acta Physiologiae Plantarum 30:545–552.

Fitter, A.H. 1987. An Architectural Approach to the Comparative Ecology of Plant Root Systems. New Phytologist 106:61-77.

Flexas, J., and H. Medrano. 2002. Drought-inhibition of Photosynthesis in C_3 Plants: Stomatal and None-stomatal Limitations Revisited. Annals of Botany 89:183-189.

Flexas, J., J. Bota, F. Loreto, G. Cornic, and T.D. Sharkey. 2004. Diffusive and Metabolic Limitations to Photosynthesis under Drought and Salinity in C_3 Plants. Plant Biology 6:269-279.

Flexas, J., J. Bota, J. Galmés, H. Medrano, and M. Ribas-Carbó. 2006a. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiologia Plantarum 127:343-352.

Flexas, J., M. Ribas-Carbó, J. Bota, J. Galmés, M. Henkle, S. Martínez-Cañellas, and H. Medrano. 2006b. Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. New Phytologist 172:73-82.

Foster, E.F., A. Pajarito, and J. Acosta-Gallegos. 1995. Moisture stress impact on N partitioning, N remobilization and N-use efficiency in beans (*Phaseolus vulgaris*). The Journal of Agricultural Science 124:27-37.

Foyer, C.H., A.J. Bloom, G. Queval, and G. Noctor. 2009. Photorespiratory Metabolism: Genes, Mutants, Energetics, and Redox Signaling. Annual Review of Plant Biology 60:455-484.

Garay, A.F., and W.W. Wilhelm. 1982. Root System Characteristics of Two Soybean Isolines Undergoing Water Stress Conditions. Agronnomy Journal 75:973-977.

Garnett, T., V. Conn, and B.N. Kaiser. 2009. Root based approaches to improving nitrogen use efficiency in plants. Plant, Cell & Environment 32:1272-1283.

Garside, A.L., R.J. Lawn, and D.E. Byth. 1992. Irrigation management of soybean (*Glycine max* (L.) Merrill) in a semi-arid tropical environment. I. Effect of irrigation frequency on growth, development and yield. Australian Journal of Agricultural Research 43:1003-1017.

Gebeyehu, S. 2006. Physiological Response to Drought Stress of Common Bean (*Phaseolus vulgaris* L.) Genotypes Differing in Drought Resistance, Justus-Liebig-Universität Giessen, Germany.

George T., P. W. Singleton, and C.v. Kessel. 1996. The use of nitrogen ^{15} natural abundance and nitrogen yield of non nodulating isolines to estimate nitrogen fixation by soybeans (*Glycine max* L.) across three elevations. Biology and Fertility of Soils 15:81-86.

Ghannoum, O. 2009. C_4 Photosynthesis and water stress. Annals of Botany 103:635-644.

Gilbert, M.E., M.A. Zwieniecki, and N.M. Holbrook. 2011. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. Journal of Experimental Botany 62:2875-2887.

Gimenez, C., V.J. Mitchell, and D.W. Lawlor. 1992. Regulation of Photosynthetic Rate of Two Sunflower Hybrids under Water Stress. Plant Physiology 98:516-524.

Gordon, A.J., F.R. Minchin, C.L. James, and O. Komina. 1999. Sucrose Synthase in Legume Nodules Is Essential for Nitrogen Fixation. Plant Physiology 120:867-878.

Graham, P.H., and C.P. Vance. 2003. Legumes: Importance and Constraints to Greater Use. Plant Physiology 131:872-877.

Gresshoff, P.M. 2010. Molecular Genetic Analysis of Nodulation Genes in Soybean, p. 275-318 Plant Breeding Reviews. John Wiley & Sons, Inc.

Grzesiak, S., W. Filek, S. Pienkowski, and B. Nizio. 1996. Screening for Drought Resistance: Evaluation of Drought Susceptibility Index of Legume Plants under Natural Growth Conditions. Journal of Agronomy and Crop Science 177:237-244.

Hall, A.E., R.A. Richards, A.G. Condon, G.C. Wright, and G.D. Farquhar. 1998. Carbon Isotope Discrimination and Plant Breeding, p. 81-113 Plant Breeding Reviews. John Wiley & Sons, Inc.

Hardarson, G., F.A. Bliss, M.R. Cigales-Rivero, R.A. Henson, J.A. Kipe-Nolt, L. Longeri, A. Manrique, J.J. Peña-Cabriales, P.A.A. Pereira, C.A. Sanabria, and S.M. Tsai. 1993. Genotypic variation in biological nitrogen fixation by common bean. Plant and Soil 152:59-70.

Hardarson G., F.A. Bliss, M.R. Cigales-Rivero, R.A. Henson, J.A. Kipe-Nolt, L. Longeri, A. Manrique, J.J. Pena-Cabriales, P.A.A. Preira, C.A.S. and, and S.M. Tsai. 1993. Genotypic variation in biological nitrogen fixation by common bean. Plant and Soil 152:59-70.

Hardy, R.W.F., R.C. Burns, and R.D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry 5:47-81.

Harmon, C. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica et Cosmochimica Acta 12:133-149.

Hay, R.K.M., and J.R. Porter. 2006. The physiology of crop yield. Scond ediition ed. Blackwell publishing Ltd.

Herridge, D.F., and S.K.A. Danso. 1995. Enhancing crop legume N_2 fixation through selection and breeding. Plant and Soil 174:51-82.

Herridge, D.F., M.B. Peoples, and R.M. Boddey. 2008. Global inputs of biological nitrogen fixation in agricultural systems Plant and Soil Volume 311, :1-18.

Hoagland, D.R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station 347:32 pp.

HÖGberg, P. 1997. Tansley Review No. 95 15N natural abundance in soil-plant systems. New Phytologist 137:179-203.

Holdensen, L., H. Hauggaard-Nielsen, and E. Jensen. 2007. Short-range spatial variability of soil δ^{15}N natural abundance – effects on symbiotic N_2-fixation estimates in pea. Plant and Soil 298:265-272.

Hubick, K., and G. Farquhar. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. Plant, Cell & Environment 12:795-804.

Hubick, K.T., G.D. Farquhar, and R. Shorter. 1986. Correlation Between Water-Use Efficiency and Carbon Isotope Discrimination in Diverse Peanut (*Arachis*) Germplasm. Functional Plant Biology 13:803-816.

Hufstetler, E.V., H.R. Boerma, T.E. Carter, and H.J. Earl. 2007. Genotypic Variation for Three Physiological Traits Affecting Drought Tolerance in Soybean. Crop Science 47:25-35.

Hungria, M., and M.A.T. Vargas. 2000. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Research 65:151-164.

Hunt, S., B.J. King, and D.B. Layzell. 1989. Effects of Gradual Increases in O_2 Concentration on Nodule Activity in Soybean. Plant Physiology. 91:315-321.

IITA. 2000. Annual report 2000 (Project 4). Improving maize-grain legume systems in west and central Africa.

IITA. 2009a. http://www.iita.org/ [Online].

IITA. 2009b. http://r4dreview.org/ [Online].

JMP®8.02. 2011. Statistical Discovery Software; SAS Institute, Inc.: Cary, NC, USA.

Johnson, D.A., and K.H. Asay. 1993. Viewpoint: Selection for Improved Drought Response in Cool-Season Grasses. Journal of Range Management 46:194-202

Johnson, D.A., and M.D. Rumbaugh. 1995. Genetic Variation and Inheritance Characteristics for Carbon Isotope Discrimination in Alfalfa. Journal of Range Management 48:126-131.

Johnson, D.A., K.H. Asay, L.L. Tieszen, J.R. Ehleringer, and P.G. Jefferson. 1990. Carbon Isotope Dicrimination: Potential in Screening Cool-Season Grasses for Water-Limited Environments. Crop Science 30:338-343.

Johnson, R.C., and L.L. Tieszen. 1994. Variation for Water-Use Efficiency in Alfalfa Germplasm. Crop Science 34:452-458.

Jongdee, B., S. Fukai, and M. Cooper. 2002. Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crops Research 76:153-163.

Jury, W.A., H.J. Vaux Jr, and L.S. Donald. 2007. The Emerging Global Water Crisis: Managing Scarcity and Conflict Between Water Users, p. 1-76 Advances in Agronomy, Vol. Volume 95. Academic Press.

Kapotis, G., G. Zervoudakis, T. Veltsistas, and G. Salahas. 2003. Comparison of Chlorophyll Meter Readings with Leaf Chlorophyll Concentration in Amaranthus vlitus: Correlation with Physiological Processes1. Russian Journal of Plant Physiology 50:395-397.

Kashiwagi, J., L. Krishnamurthy, J.H. Crouch, and R. Serraj. 2006. Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. Field Crops Research 95:171-181.

Khazaei, H., S.-D. Mohammady, M. Zaharieva, and P. Monneveux. 2009. Carbon isotope discrimination and water use efficiency in Iranian diploid, tetraploid and hexaploid wheats grown under well-watered conditions. Genetic Resources and Crop Evolution 56:105-114.

Kim, T.H., Böhmer Maik , H. Hu, N. Nishimura, and J.I. Schroeder. 2010. Guard Cell Signal Transduction Network: Advances in Understanding Abscisic Acid, $C0_2$, and Ca^{2+} Signaling. Annual Review of Plant Biology.

King, C.A., and L.C. Purcell. 2001. Soybean Nodule Size and Relationship to Nitrogen Fixation Response to Water Deficit. Crop Science 41:1099-1107.

King, C.A., and L.C. Purcell. 2005. Inhibition of N2 Fixation in Soybean Is Associated with Elevated Ureides and Amino Acids. Plant Physiology 137:1389-1396.

King, C.A., and L.C. Purcell. 2006. Genotypic Variation for Shoot N Concentration and Response to Water Deficits in Soybean. Crop Science 46:2396-2402.

Kirda, C., S. Danso, and F. Zapata. 1989. Temporal water stress effects on nodulation, nitrogen accumulation and growth of soybean. Plant and Soil 120:49-55.

Knight, J., F. Verhees, C. Van Kessel, and A. Slinkard. 1993. Does carbon isotope discrimination correlate with biological nitrogen fixation? Plant and Soil 153:151-153.

Kobata, T., T. Okuno, and T. Yamamoto. 1996. Contributions of capacity for soil water extraction and water use efficiency to maintenance of dry matter production in rice subjected to drought. Japan Journal of Crop Science 65 652–662.

Kohi, D.H., G. Shearer, and J.E. Harper. 1980. Estimates of N_2 Fixation Based on Differences in the Natural Abundance of ^{15}N in Nodulating and Nonnodulating Isolines of Soybeans. Plant Physiology 66:61-65.

Kondo, M., P. Pablico, D. Aragones, and R. Agbisit. 2004. Genotypic variations in carbon isotope discrimination, transpiration efficiency, and biomass production in rice as affected by soil water conditions and N. Plant and Soil 267:165-177.

Kumarasinghe, K.S., C. Kirda, A.R.A.G. Mohamed, F. Zapata, and S.K.A. Danso. 1992. ^{13}C isotope discrimination correlates with biological nitrogen fixation in soybean (*Glycine max* (L.) Merrill) Plant and Soil 139:145-147.

Ladrera, R., D. Marino, E. Larrainzar, E.M. González, and C. Arrese-Igor. 2007. Reduced Carbon Availability to Bacteroids and Elevated Ureides in Nodules, But Not in Shoots, Are Involved in the Nitrogen Fixation Response to Early Drought in Soybean. Plant Physiology 145:539-546.

Larcher, W. 2003. Physiological plant ecology: ecophysiology and stress physiology of functional groups. Fourth ed. Springer, Germany

Lawlor, D.W. 2002a. Limitation to Photosynthesis in Water-stressed Leaves: Stomata vs. Metabolism and the Role of ATP. Annals of Botany 89:871-885.

Lawlor, D.W. 2002b. Limitation to Photosynthesis in Water-stressed Leaves: Stomata. Annals of Botany 89:871-885.

Lawlor, D.W., and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell & Environment 25:275-294.

Lawlor, D.W., and W. Tezara. 2009a. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany.

Lawlor, D.W., and W. Tezara. 2009b. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany 103:561-579.

Lawn, R.J., and A.T. James. 2011. Application of physiological understanding in soybean improvement. I. Understanding phenological constraints to adaptation and yield potential. Crop and Pasture Science 62:1-11.

Leport, L., N.C. Turner, R.J. French, M.D. Barr, R. Duda, S.L. Davies, D. Tennant, and K.H.M. Siddique. 1999. Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. European Journal of Agronomy 11:279-291.

Li, X., Y. Feng, and L. Boersma. 1994. Partition of photosynthates between shoot and root in spring wheat (*Triticum aestivum* L.) as a function of soil water potential and root temperature. Plant and Soil 164:43-50.

Li, Z., P. Mu, C. Li, H. Zhang, Z. Li, Y. Gao, and X. Wang. 2005. QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland *japonica* rice in three environments. TAG Theoretical and Applied Genetics 110:1244-1252.

Liang, Y., and J.M. Harris. 2005. Response of root branching to abscisic acid is correlated with nodule formation both in legumes and nonlegumes. American Journal of Botany 92:1675-1683.

Liang, Y., D.M. Mitchell, and J.M. Harris. 2007. Abscisic acid rescues the root meristem defects of the *Medicago truncatula* latd mutant. Developmental Biology 304:297-307.

Liu, F., M.N. Andersen, S.-E. Jacobsen, and C.R. Jensen. 2005. Stomatal control and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. Environmental and Experimental Botany 54:33-40.

Lopes, M.S., J.L. Araus, P.D.R. van Heerden, and C.H. Foyer. 2011. Enhancing drought tolerance in C_4 crops. Journal of Experimental Botany 62:3135-3153.

Lucas, M., C. Godin, C. Jay-Allemand, and L. Laplaze. 2008. Auxin fluxes in the root apex co-regulate gravitropism and lateral root initiation. Journal of Experimental Botany 59:55-66.

Lynch, J. 1995. Root Architecture and Plant Productivity. Plant Physiology 109:7-13.

Lynch, J., and K. Brown. 2001. Topsoil foraging - an architectural adaptation of plants to low phosphorus availability. Plant and Soil 237:225-237.

Lynch, J., M. Ho, and L. phosphorus. 2005. Rhizoeconomics: Carbon costs of phosphorus acquisition. Plant and Soil 269:45-56.

Lynch, J.P. 2007. TURNER REVIEW No. 14 Roots of the Second Green Revolution. Australian Journal of Botany 55:493-512.

Lynch, J.P. 2011. Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for Future Crops. Plant Physiology 156:1041-1049.

Malamy, J.E. 2005. Intrinsic and environmental response pathways that regulate root system architecture. Plant, Cell & Environment 28:67-77.

Manavalan, L.P., S.K. Guttikonda, L.-S. Phan Tran, and H.T. Nguyen. 2009. Physiological and Molecular Approaches to Improve Drought Resistance in Soybean. Plant and Cell Physiology 50:1260-1276.

Mario Valenzuela-Vazquez , Artemio Escobedo-Mendoza, Jose Luis Almanza-Sandoval , and Asunción Ríos-Torres. 1997. Pressure bomb. Final report: New Mexico state University

Markwell, J., J. Osterman, and J. Mitchell. 1995. Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynthesis Research 46:467-472.

Martin, B., and Y.R. Thorstenson. 1988. Stable Carbon Isotope Composition ($d^{13}C$), Water Use Efficiency, and Biomass Productivity of *Lycopersicon esculentum*, *Lycopersicon pennellii*, and the F1 Hybrid. Plant Physiology 88:213-217.

Matamoros, M.A., L.M. Baird, P.R. Escuredo, D.A. Dalton, F.R. Minchin, I. Iturbe-Ormaetxe, M.C. Rubio, J.F. Moran, A.J. Gordon, and M. Becana. 1999. Stress-Induced Legume Root Nodule Senescence. Physiological, Biochemical, and Structural Alterations. Plant Physiology 121:97-112.

Mayers, J.D., R.J. Lawn, and D.E. Byth. 1991. Adaptation of soybean (*Glycine max* (L.) Merrill) to the dry season of the tropics. II. Effects of genotype and environment on biomass and seed yield. Australian Journal of Agricultural Research 42:517-530.

McCoy, E.L., L. Boersma, and M. Ekasingh. 1990. Net Carbon Allocation in Soybean Seedlings as Influenced by Soil Water Stress at Two Soil Temperatures. Botanical Gazette 151:497-505.

Medrano, H., J.M. Escalona, J. Bota, J. GulÃ□As, and J. Flexas. 2002. Regulation of Photosynthesis of C_3 Plants in Response to Progressive Drought: Stomatal Conductance as a Reference Parameter. Annals of Botany 89:895-905.

Mencuccini, M., S. Mambelli, and J. Comstock. 2000. Stomatal responsiveness to leaf water status in common bean (*Phaseolus vulgaris* L.) is a function of time of day. Plant, Cell & Environment 23:1109-1118.

Menéndez, C.M., and A.E. Hall. 1996. Heritability of Carbon Isotope Discrimination and Correlations with Harvest Index in Cowpea. Crop Science 36:233-238.

Merah, O., E. Deléens, and P. Monneveux. 2001. Relationships between carbon isotope discrimination, dry matter production, and harvest index in durum wheat. Journal of Plant Physiology 158:723-729.

Minchin, F.R. 1997. Regulation of oxygen diffusion in legume nodules. Soil Biology and Biochemistry 29:881-888.

Minolta. 1990. Specifications in detail—Chlorophyll meter SPAD 502. Minolta Technical Note TE102-601-01.

Miyashita, K., S. Tanakamaru, T. Maitani, and K. Kimura. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environmental and Experimental Botany 53:205-214.

Mohamed, M., N. Keutgen, AA. Tawfik, and G. Noga. 2002. Dehydration-avoidance responses of tepary bean lines differing in drought resistance. Journal of Plant Physiology 159:31–38.

Morgan, J.A., D.R. LeCain, T.N. McCaig, and J.S. Quick. 1993. Gas Exchange, Carbon Isotope Discrimination, and Productivity in Winter Wheat. Crop Science 33:178-186.

Morgan, J.M. 1992. Adaptation to water deficits in three grain legume species. Mechanisms of turgor maintenance. Field Crops Research 29:91-106.

Morgan, J.M., and A.G. Condon. 1986. Water Use, Grain Yield, and Osmoregulation in Wheat. Functional Plant Biology 13:523-532.

Mostajeran, A., and Z. Rengel. 2007. Correlation Between the Carbon Isotope Composition ($d^{13}C$) of *Puccinellia ciliata* and *Balansa Clover* in Duel Stresses of Waterlogging and Salinity (NaCl). Scientia Iranica 327-335.

Müller, P., X.-P. Li, and K.K. Niyogi. 2001. Non-Photochemical Quenching. A Response to Excess Light Energy. Plant Physiology 125:1558-1566.

Munn, S. Bosch, and L. Alegre. 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. Functional Plant Biology 31:203-216.

Muñoz-Perea, C., R. Allen, D. Westermann, J. Wright, and S. Singh. 2007. Water use efficiency among dry bean landraces and cultivars in drought-stressed and non-stressed environments. Euphytica 155:393-402.

Negash, K., S. Genbeyehu, K. Tumsa, and E. Habte. 2011. Enhancing. Progress and Prospects with research and development of TLII in Ethiopia, 3rd ESA Annual Review & Planning Meeting;22-26 August 2011, Lilongwe, Malawi.

Nguyen, H.T., R.C. Babu, and A. Blum. 1997. Breeding for Drought Resistance in Rice: Physiology and Molecular Genetics Considerations. Crop Science 37:1426-1434.

Nibau, C., D.J. Gibbs, and J.C. Coates. 2008. Branching out in new directions: the control of root architecture by lateral root formation. New Phytologist 179:595-614.

Nielsen, K.L., J.P. Lynch, and H.N. Weiss. 1997. Fractal Geometry of Bean Root Systems: Correlations between Spatial and Fractal Dimension. American Journal of Botany 84:26-33.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. Phytochemistry 20:553-567.

O'Leary, M.H. 1988. Carbon Isotopes in Photosynthesis. BioScience 38:328-336

O'Toole, J.C., and S.K. De Datta. 1986. Drought resisistance in rainfed lowland rice. Progress in rainfed rice. IRRI, Los Banos, Philippines, pp 145-158.

Ort, D., O. K, and W. RR. 1994. Depression of photosynthesis in crops with water deficits. In: Baker NR, Bowyer JR, eds. Photoinhibition of photosynthesis from molecular mechanism to the field., p. 315-329. Oxford: BIOS Science Publishers Ltd, .

Osmond, B., M. Badger, K. Maxwell, O. Bjorkman, and R. Leegood. 1997. Too many photos: photorespiration, photoinhibition and photooxidation. Trends Plant Science 2:119-121.

Osmont, K.S., R. Sibout, and C.S. Hardtke. 2007. Hidden Branches: Developments in Root System Architecture. Annual Review of Plant Biology 58:93-113.

Pantuwan, G., S. Fukai, M. Cooper, S. Rajatasereekul, and J.C. O'Toole. 2002. Yield response of rice (*Oryza sativa* L.) genotypes to different types of drought under rainfed lowlands: Part 1. Grain yield and yield components. Field Crops Research 73:153-168.

Parry, M.A.J., P.J. Andralojc, S. Khan, P.J. Lea, and A.J. Keys. 2002. Rubisco Activity: Effects of Drought Stress. Annals of Botany 89:833-839.

Pask, A., J. Pietragalla, DM. Mullan, and MP. Reynolds (Eds.). 2012. Physiological Breeding II: A Field Guide to Wheat Phenotyping. :CIMMYT, Mexico, D.F.

Passioura, J.B. 1996. Drought and drought tolerance. Plant Growth Regulation 20:79-83.

Pastenes, C., P. Pimentel, and J. Lillo. 2005. Leaf movements and photoinhibition in relation to water stress in field-grown beans. Journal of Experimental Botany 56:425-433.

Pate, J.S., C.A. Atkins, D.B. Layzell, and B.J. Shelp. 1984. Effects of N_2 Deficiency on Transport and Partitioning of C and N in a Nodulated Legume. Plant Physiology 76:59-64.

Pazdernik, D.L., P.H. Graham, and J.H. Orf. 1997. Heritability in the early nodulation of F_3 and F_4 soybean lines. Canadian Journal of Plant Science 77:201-205.

Pazdernik, D.L., P.H. Graham, C.P. Vance, and J.H. Orf. 1996. Host Genetic Variation in the Early Nodulation and Dinitrogen Fixation of Soybean. Crop Science 36:1102-1107.

Peña-Cabriales J., and J. Castellanos. 1993. Effect of water stress on N2 fixation and grain yield of Phaseolus vulgaris L. . Plant Soil 152.

Peoples, M.B., and D.F. Herridge. 1990. Nitrogen fixation by legumes in tropical and subtropical agriculture CSIRO Division of Plant Industry, Canberra, A.C.T., Australia.

Peoples, M.B., U. Murray John, and H. David. 2009. Measuring symbiotic nitrogen fixation by legumes. In: Nitrogen fixation in crop production / D. W. Emerich and H. B. Krishnan [eds], pp.125-170 American Society of Agronomy.

Peoples, M.B., A.W. Faizah, B. Rerkasem, and D.F. Herridge. 1989. Methods for evaluating nitrogen fixation by nodulated legumes in the field Australian Centre for International Agricultural Research, Canberra, Australia.

Pfündel, E., and W. Bilger. 1994. Regulation and possible function of the violaxanthin cycle. Photosynth Research 42:89-109.

Pinheiro, H., F. Damatta, A.M. Chaves, M. Loureiro, and C. Ducatti. 2005. Drought Tolerance is Associated with Rooting Depth and Stomatal Control of Water Use in Clones of Coffea canephora. Annals of Botany 96:101-108.

Plies, B.E., T. Kong, S. Schubert, and K. Mengel. 1995. Effect of water stress on plant growth, nitrogenase activity and nitrogen economy of four different cultivars of *Vicia faba* L Elsevier, Amsterdam, PAYS-BAS.

Purcell, L.C., and T.R. Sinclair. 1994. An osmotic hypothesis for the regulation of oxygen permeability in soybean nodules. Plant, Cell & Environment 17:837-843.

Purcell, L.C., and T.R. Sinclair. 1995. Nodule gas exchange and water potential response to rapid imposition of water deficit. Plant, Cell & Environment 18:179-187.

Rabha, B.K., and D.C. Uprety. 1998. Effects of Elevated CO_2 and Moisture Stress on *Brassica Juncea*. Photosynthetica 35:597-602.

Rainbird, R.M., J.H. Thorne, and R.W.F. Hardy. 1984. Role of Amides, Amino Acids, and Ureides in the Nutrition of Developing Soybean Seeds. Plant Physiology 74:329-334.

Ramirez-Vallejo, P., and J. Kelly. 1998. Traits related to drought resistance in common bean. Euphytica 99:127-136.

Ramos, M.L.G., A.J. Gordon, F.R. Minchin, J.I. Sprent, and R. Parsons. 1999. Effect of Water Stress on Nodule Physiology and Biochemistry of a Drought Tolerant Cultivar of Common Bean (*Phaseolus vulgaris* L.). Annals of Botany 83:57-63.

Raven, J.A., and G.D. Farquhar. 1990. The influence of N metabolism and organic acid synthesis on the natural abundance of isotopes of carbon in plants. New Phytologist 116:505-529.

Rebetzke, G., R. Richards, A. Condon, and G. Farquhar. 2006. Inheritance of Carbon Isotope Discrimination in Bread Wheat (*Triticum Aestivum* L.). Euphytica 150:97-106.

Regent Instruments Inc. 2011. WinRHIZO for root analysis: www.regentinstruments.com [Online].

Reynolds, J., and J. Chen. 1996. Modelling whole-plant allocation in relation to carbon and nitrogen supply: Coordination versus optimization: Opinion. Plant and Soil 185:65-74.

Reynolds, M., F. Dreccer, and R. Trethowan. 2007. Drought-adaptive traits derived from wheat wild relatives and landraces. Journal of Experimental Botany 58:177-186.

Ribas-Carbo, M., N.L. Taylor, L. Giles, S. Busquets, P.M. Finnegan, D.A. Day, H. Lambers, H.l. Medrano, J.A. Berry, and J. Flexas. 2005. Effects of Water Stress on Respiration in Soybean Leaves. Plant Physiology 139:466-473.

Ronis, D.H., D.J. Sammons, W.J. Kenworthy, and J.J. Meisinger. 1985. Heritability of Total and Fixed N Content of the Seed in Two Soybean Populations. Crop Science 25:1-4.

Rytter, R.M. 2005. Water Use Efficiency, Carbon Isotope Discrimination and Biomass Production of Two Sugar Beet Varieties Under Well-Watered and Dry Conditions. Journal of Agronomy and Crop Science 191:426-438.

Sall, K., and T.R. Sinclair. 1991. Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. Plant and Soil 133:31-37.

Saranga, Y., I. Flash, and D. Yakir. 1998. Variation in Water-Use Efficiency and Its Relation to Carbon Isotope Ratio in Cotton. Crop Science 38:782-787.

Schubert, S., R. Serraj, E. Plies-Balzer, and K. Mengel. 1995. Effect of Drought Stress on Growth, Sugar Concentrations and Amino Acid Accumulation in N2-Fixing Alfalfa (*Medicago sativa*). Journal of Plant Physiology 146:541-546.

Serraj, R., T.R. Sinclair, and L.C. Purcell. 1999. Symbiotic N_2 fixation response to drought. Journal of Experimental Botany 50:143-155.

Serraj, R., P. Fleurat-Lessard, B. Jaillard, and J.J. Drevon. 1995. Structural changes in the innercortex cells of soybean root nodules are induced by short-term exposure to high salt or oxygen concentrations. Plant, Cell & Environment 18:455-462.

Serraj, R., S. Bona, L.C. Purcell, and T.R. Sinclair. 1997. Nitrogen accumulation and nodule activity of field-grown 'Jackson' soybean in response to water deficits. Field Crops Research 52:109-116.

Shaheen, R., and R.C. Hood-Nowotny. 2005. Effect of drought and salinity on carbon isotope discrimination in wheat cultivars. Plant Science 168:901-909.

Shearer, G., and D.H. Kohl. 1986. N_2-Fixation in Field Settings: Estimations Based on Natural ^{15}N Abundance. Functional Plant Biology 13:699-756.

Shenkut, A.A., and M.A. Brick. 2003. Traits associated with dry edible bean (*Phaseolus vulgaris* L.) productivity under diverse soil moisture environments. Euphytica 133:339-347.

Sheshshayee, M.S., H. Bindumadhava, R. Ramesh, T.G. Prasad, M.R. Lakshminarayana, and M. Udayakumar. 2005. Oxygen isotope enrichment ($\Delta^{18}O$) as a measure of time-averaged transpiration rate. Journal of Experimental Botany 56:3033-3039.

Siddique, K.H.M., S.P. Loss, K.L. Regan, and R. Jettner. 1999. Adaptation of cool season grain legumes in Mediterranean-type environments of south-western Australia. Australian Journal of Agricultural Research 50:375-387.

Siddique, K.H.M., K.L. Regan, D. Tennant, and B.D. Thomson. 2001. Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. European Journal of Agronomy 15:267-280.

Siddique, M.R.B., A. Hamid, and M.S. Islam. 2000. Deought stress effects on water relations on wheat. Botanical Bulletin of Academia Sinica 41 35-39.

Signarbieux, C., and U. Feller. 2011. Non-stomatal limitations of photosynthesis in grassland species under artificial drought in the field. Environmental and Experimental Botany 71:192-197.

Silsbury, J.H. 1977. Energy requirement for symbiotic nitrogen fixation. Nature 267:149-150.

Sincik, M., B.N. Candogan, C. Demirtas, H. Buyukcangaz, S. Yazgan, and T. Goksoy. 2008. Degficit Irrigation of Soya Bean (*Glyine max* (L) Merr.) in a Sub-humid Climates Journal of Agronomy and Crop Science 194:200-205.

Sinclair, T.R. 1986. Water and nitrogen limitations in soybean grain production I. Model development. Field Crops Research 15:125-141.

Sinclair, T.R., and R. Serraj. 1995. Dinitrogen fixation sensitivity to drought among grain legume species. Nature 378 344.

Sinclair, T.R., and R.C. Muchow. 2001. System Analysis of Plant Traits to Increase Grain Yield on Limited Water Supplies. Agronomy Journal 93:263–270.

Sinclair, T.R., L.C. Purcell, C.A. King, C.H. Sneller, P. Chen, and V. Vadez. 2007. Drought tolerance and yield increase of soybean resulting from improved symbiotic N2 fixation. Field Crops Research 101:68-71.

Singh, V., E. Oosterom, D. Jordan, C. Messina, M. Cooper, and G. Hammer. 2010. Morphological and architectural development of root systems in sorghum and maize. Plant and Soil 333:287-299.

Smeal, D., and H. Zhang. 1994. Chlorophyll meter evaluation for nitrogen management in corn. Communications in Soil Science and Plant Analysis 25:1495-1503.

Soares-Cordeiro, A.S., S.P. Driscoll, T.K. Pellny, E. Olmos, M.C. ArrabaÇA, and C.H. Foyer. 2009a. Variations in the dorso-ventral organization of leaf structure and Kranz anatomy coordinate the control of photosynthesis and associated signalling at the whole leaf level in monocotyledonous species. Plant, Cell & Environment 32:1833-1844.

Soares-Cordeiro, A.S., A.E. Carmo-Silva, A. Bernardes da Silva, J. Marques da Silva, A.J. Keys, and M.C. Arrabaça. 2009b. Effects of rapidly imposed water deficit on photosynthetic parameters of three C4 grasses. Photosynthetica 47:304-308.

Sponchiado, B.N., J.W. White, J.A. Castillo, and P.G. Jones. 1989. Root Growth of Four Common Bean Cultivars in Relation to Drought Tolerance in Environments with Contrasting Soil Types. Experimental Agriculture 25:249-257.

Sponchiado BN, JW White, J.C. and, and P. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Experimental Agriculture 25:249–257.

Steele, K.A., D.S. Virk, R. Kumar, S.C. Prasad, and J.R. Witcombe. 2007. Field evaluation of upland rice lines selected for QTLs controlling root traits. Field Crops Research 101:180-186.

Streeter, J.G. 2003. Effects of drought on nitrogen fixation in soybean root nodules. Plant, Cell & Environment 26:1199-1204.

Subbarao, G.V., C. Johansen, A.E. Slinkard, R.C. Nageswara Rao, N.P. Saxena, Y.S. Chauhan, and R.J. Lawn. 1995. Strategies for Improving Drought Resistance in Grain Legumes. Critical Reviews in Plant Sciences 14:469-523.

Tefera, H. 2011. Breeding for Promiscuous Soybeans at IITA, Soybean - Molecular Aspects of Breeding, Aleksandra Sudaric (Ed.), InTech. .

Teulat, B., O. Merah, and D. This. 2001. Carbon Isotope Discrimination and Productivity in Field-Grown Barley Genotypes. Journal of Agronomy and Crop Science 187:33-39.

Tezara, W., V.J. Mitchell, S.D. Driscoll, and D.W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401:914-917.

Tezara, W., V. Mitchell, S.P. Driscoll, and D.W. Lawlor. 2002. Effects of water deficit and its interaction with CO_2 supply on the biochemistry and physiology of photosynthesis in sunflower. Journal of Experimental Botany 53:1781-1791.

Tezara, W., D. Martinez, E. Rengifo, and A.N.A. Herrera. 2003. Photosynthetic Responses of the Tropical Spiny Shrub *Lycium nodosum* (Solanaceae) to Drought, Soil Salinity and Saline Spray. Annals of Botany 92:757-765.

Thavarajah, D., and R.A. Ball. 2006. Drought-induced changes in free amino acid and ureide concentrations of nitrogen-fixing chickpea. Canadian Journal of Plant Science 86:149-156.

Thomas, M.J. Robertson, S. Fukai, and M.B. Peoples. 2004. The effect of timing and severity of water deficit on growth, development, yield accumulation and nitrogen fixation of mungbean. Field Crops Research 86:67-80.

Thomson, B.D., K.H.M. Siddique, M.D. Barr, and J.M. Wilson. 1997. Grain legume species in low rainfall mediterranean-type environments I. Phenology and seed yield. Field Crops Research 54:173-187.

Trachsel, S., S. Kaeppler, K. Brown, and J. Lynch. 2011. Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. Plant and Soil 341:75-87.

Troughton, J.H., K.A. Card, and C.H. Hendy. 1974. Photosynthetic pathways and carbon isotope discrimination by plants. Carnegie Inst. Wash,Yearbook 73,768-180

Tschoep, H. 2010. The response of growth and primary metabolism to a mild but sustained nitrogen limitation in Arabidopsis thaliana. PhD dissertation. Mathematisch-Naturwissenschaftliche Fakultät, University of Potsdam, Germany.

Turner, L., and H . Gibson. 1980. Measurement of nitrogen fixation by indirect means. In Methods for Evaluating Biological Nitrogen Fixation. Edited by F. J. Bergersen. Chichester: Wiley:111 - 138.

Turner, L., and H. Gibson. 1980. Measurement of nitrogen fixation by indirect means. In Methods for Evaluating Biological Nitrogen Fixation.(ed. F.J. Bergersen),pp.111-138 Wiley, Chichester.

Turner, N.C. 1982. The role of shoot characteristics in drought tolerance of crop plants. In: Drought tolerance in crop with emphasis on rice. International rice Research Institute, Los Banos, Manila, Philippines, pp.115-134.

Turner, N.C., and N.C. Brady. 1986. Crop Water Deficits: A Decade of Progress, p. 1-51 Advances in Agronomy, Vol. Volume 39. Academic Press.

Turner, N.C., G.C. Wright, and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water-limited environments, p. 193-231 Advances in Agronomy, Volume 71. Academic Press.

Uddling, J., J. Gelang-Alfredsson, K. Piikki, and H. Pleijel. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynthesis Research 91:37-46.

Udensi, O., E.V. Ikpeme, A.A. Markson, E.A.B. Edu, E.J. Umana, and I.S. Urua. 2010. Selection of soybean genotypes using morphological markers International Journal of Current Research 7:005-008.

Unkovich, M.J., and J.S. Pate. 2000. An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. Field Crops Research 65:211-228.

Unkovich, M.J., J.S. Pate, P. Sanford, and E.L. Armstrong. 1994. Potential precision of the $\delta^{15}N$ natuural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. Australian Journal of Agricultural Research 45:119-132.

USDA. 2011. http://soils.usda.gov/ [Online].

Valles-De La Mora, B., G. Cadisch, and A. Aluja-Schunemann. 2003. Comparación de metodologías de isótopos para evaluar fijación de n atmosférico y su destino en suelos y plantas. Agrociencia 37:117-128.

Van Heerden, P.D.R., M. De Beer, D.J. Mellet, H.S. Maphike, and W. Foit. 2007. Growth media effects on shoot physiology, nodule numbers and symbiotic nitrogen fixation in soybean. South African Journal of Botany 73:600-605.

Van Heerden, P.D.R., G. Kiddle, T.K. Pellny, P.W. Mokwala, A. Jordaan, A.J. Strauss, M. de Beer, U. Schluter, K.J. Kunert, and C.H. Foyer. 2008. Regulation of Respiration and the Oxygen Diffusion Barrier in Soybean Protect Symbiotic Nitrogen Fixation from Chilling-Induced Inhibition and Shoots from Premature Senescence. Plant Physiol. 148:316-327.

Vance, C.P., K.L.M. Boylan, C.A. Maxwell, G.H. Heichel, and L.H. Leland. 1985. Transport and Partitioning of CO_2, Fixed by Root Nodules of Ureide and Amide Producing Legumes. Plant Physiology 78:774-778.

Vassey, T.L., and T.D. Sharkey. 1989. Mild Water Stress of Phaseolus vulgaris Plants Leads to Reduced Starch Synthesis and Extractable Sucrose Phosphate Synthase Activity. Plant Physiology 89:1066-1070.

Venkateswarlu, B., N. Saharan, and M. Maheswari. 1990. Nodulation and N_2 (C_2H_2) fixation in cowpea and groundnut during water stress and recovery. Field Crops Research 25:223-232.

Voisin, A.S., C. Salon, C. Jeudy, and F.R. Warembourg. 2003. Root and Nodule Growth in *Pisum sativum* L. in Relation to Photosynthesis: Analysis Using 13C-labelling. Annals of Botany 92:557-563.

Vos, J., P.E.L.v.d. Putten, and C.J. Birch. 2005. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). Field Crops Research 93:64-73.

Walsh, K.B., M.J. Canny, and D.B. Layzell. 1989. Vascular transport and soybean nodule function: II. A role for phloem supply in product export. Plant, Cell & Environment 12:713-723.

Wani, S.P., O. P. Rupela, and K. K. Lee. 1995. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. Plant and Soil 174:29-49.

Warren, C.R. 2008. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO. Journal of Experimental Botany 59:1475-1487.

White, D.S., M.J. Bell, and G.C. Wright. 1996 The potential to use carbon isotope discrimination as a selection tool to improve water use efficiency in soybean. Proceeding of the 8th Australian agronomy conference, Toowoomba, Queensland.

White, J.W., and S.P. Singh. 1991a. Breeding for Addaption to drought. In: (Schoonhoven, and A. Voysest, O., eds.) Common beans: research for crop improvement C.A.B. International in association with Centro Internacional de Agricultura Tropical.

White, J.W., and J.s.A. Castillo. 1991. Evaluation of Diverse Shoot Genotypes on selected Root Genotypes of Common Bean under Soil Water Deficits. Crop Science 32:762-765.

White, J.W., and S.P. Singh. 1991b. Sources and inheritance of earliness in tropically adapted indeterminate common bean. Euphytica 55:15-19.

White, J.W., J.A. Castillo, and J. Ehleringer. 1990. Associations Between Productivity, Root Growth and Carbon Isotope Discrimination in (*Phaseolus vulgaris* L.) Under Water Deficit. Functional Plant Biology 17:189-198.

Whitmore, J.S. 2000. Drought Management on Farmland Springer.

Wilhite, D.A., and M.H. Glantz. 1985. Understanding: the Drought Phenomenon: The Role of Definitions. Water International 10:111-120.

Wilson, J.B. 1988. A Review of Evidence on the Control of Shoot: Root Ratio, in Relation to Models. Annals of Botany 61:433-449.

Wortmann, C.S., R.A. Kirkby, C.A.E. and, and D.J. Allen. 1998. Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa . Cali, Colombia: Centro Internacional de Agricultura Tropical. CIAT publication: no. 297.

Xiong, L., R.-G. Wang, G. Mao, and J.M. Koczan. 2006. Identification of Drought Tolerance Determinants by Genetic Analysis of Root Response to Drought Stress and Abscisic Acid. Plant Physiology 142:1065-1074.

Yadav, R., B. Courtois, N. Huang, and G. McLaren. 1997. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. TAG Theoretical and Applied Genetics 94:619-632.

Yamamoto, A., T. Nakamura, J.J. Adu-Gyamfi, and M. Saigusa. 2002. Relationship between chlorophyll content in leaves of sorghum and pigeon pea determined by extraction method by chlorophyll meter (SPAD-502) Journal of Plant Nutrition 25:2295-2301.

Yoneyama, T., and T. Ohtani. 1983. Variations of Natural 13C Abundances in Leguminous Plants. Plant and Cell Physiology 24:971-977.

Yordanov, I., D. Stefanov, V. Krasteva, M. Gourmanova, and V. Goltsev. 2012. Drought stress responses in plants - molecular biology, physiology and agronomical aspects 4:7-20.

Zacharisen, M.H., M.A. Brick, A.G. Fisher, J.B. Ogg, and J.R. Ehleringer. 1999. Relationships between productivity and carbon isotope discrimination among dry bean lines and F_2 progeny. Euphytica 105:239-250.

Zahran, H.H. 1999a. Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. Microbiology and Molecular Biology Reviews 968–989.

Zahran, H.H. 1999b. Rhizobium-Legume Symbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate. Microbiology and Molecular Biology Reviews 63:968-989.

Zhang, X., N. Wu, and C. Li. 2005. Physiological and growth responses of *Populus davidiana* ecotypes to different soil water contents. Journal of Arid Environments 60:567-579.

Zhao, J., J. Fu, H. Liao, Y. He, H. Nian, Y. Hu, L. Qiu, Y. Dong, and X. Yan. 2004. Characterization of root architecture in an applied core collection for phosphorus efficiency of soybean germplasm. Chinese Science Bulletin 49:1611-1620.

Zhu, J.-K. 2001. Cell signaling under salt, water and cold stresses. Current Opinion in Plant Biology 4:401-406.

Zhu, J.-K. 2002. Salt and Drought Stress Signal Transduction in Plants. Annual Review of Plant Biology 53:247-273.

Zhu, X.-G., S.P. Long, and D.R. Ort. 2010. Improving Photosynthetic Efficiency for Greater Yield. The Annual Review of Plant Biology 61:235-261.

APPENDIX

Appendix 1: Analysis of variance comparisons of the effects of drought on photosynthetic CO_2 assimilation (A), stomatal conductance (G), Fv/Fm ratios and intracellular CO_2 concentrations (Ci) in the soybean cultivars: Prima 2000, A-5409RG and Jackson. Data for days 0, 7, 14 and 18 of the experiment were used in this analysis.

Variation	**d.f.**	**Means square**			
		A	**G**	**Fv/Fm**	**Ci**
Cultivar (C)	2	20.09 *	6.74 **	0.070 **	15272.4 **
Drought (D)	1	1065.98 **	68.12 **	0.091 **	376790.8 **
C*D	2	63.98 *	4.95 **	0.0156 *	1878.6 ns
Experimental error	117	5.88	0.244	0.002	1536.4
Total d.f.	122				

* $P < 0.05$, and ** $P < 0.01$, d.f. degrees of freedom

Appendix 2: Analysis of variance for six common bean lines on CO_2 assimilation (μmol $m^{-2}s^{-1}$) **(A)**, stomatal conductance (mmol $m^{-2}s^{-1}$) (G), leaf, stem and root dry mass (DW), nodule fresh mass (FW) and symbiotic nitrogen fixation (SNF/g of nodules) estimated by acetylene reduction assay, under drought and well- watered conditions for 18 days.

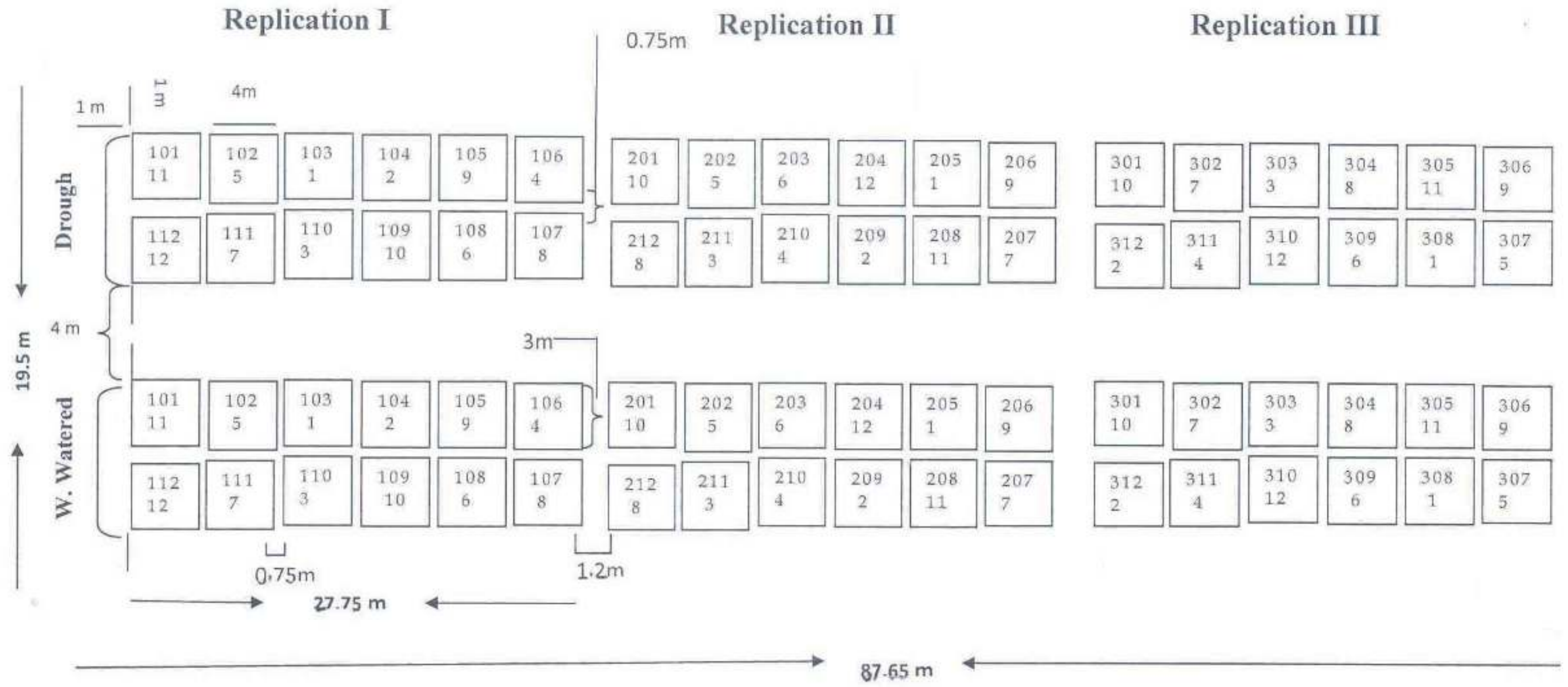
Variation	**d.f.**	**Means square**						
		A	**G**	**Leaf DW**	**Stem DW**	**Root DW**	**Nodule FW**	**SNF**
Well-watered								
Line	5	17.761	210154	1.13	2.280	1.768	1.34	1.954
P-value		0.027	0.012	0.003	0.008	<.0001	<.0001	<.0001
Significance		*	*	*	**	**	ns	**
Drought								
Line	5	51.253	79162	1.032	1.10	5.024	0.755	1.425
P-value		0.037	0.043	0.004	<.0001	0.002	0.0465	0.0067
Significance		*	*	**	**	**	*	**
Exp. Error	84	20.64	43847.1	0.276	0.184	1.223	0.292	0.357
Total	89							

* and ** indicates significance at $P \leq 0.05$, and 0.01, respectively, ns = non-significant

Appendix 3: List of treatments and randomization of bean lines in a randomized complete block design with field layout

Treatment no.	**Lines**	**Replications**		
		I	**II**	**III**
1	BT _6-1-1	103	205	308
2	BT _34-1-1	104	209	312
3	BT _51-1-1	110	211	303
4	BT _147-3	106	210	311
5	DOR 364	102	202	307
6	BAT 477	108	203	309
7	DOR 364-NN	111	207	302
8	BAT 477-NN	107	212	304
9	A5409RG	105	206	306
10	Jackson	109	201	301
11	Prima 2000	101	208	305
12	PAN 185	112	204	310

101 -112, 201 -212 and 301-312, represents plot number for replication one, two and three respectively.



Drough

101 11	102 5	103 1	104 2	105 9	106 4	201 10	202 5	203 6	204 12	205 1	206 9	301 10	302 7	303 3	304 8	305 11	306 9
112 12	111 7	110 3	109 10	108 6	107 8	212 8	211 3	210 4	209 2	208 11	207 7	312 2	311 4	310 12	309 6	308 1	307 5

W. Watered

101 11	102 5	103 1	104 2	105 9	106 4	201 10	202 5	203 6	204 12	205 1	206 9	301 10	302 7	303 3	304 8	305 11	306 9
112 12	111 7	110 3	109 10	108 6	107 8	212 8	211 3	210 4	209 2	208 11	207 7	312 2	311 4	310 12	309 6	308 1	307 5

Appendix 4: Analysis of variance for nine bean lines for root morphology traits under drought and well-watered conditions. Exposure to drought was for one month using four replicates from each treatment for acquired soil core up to 60 cm soil depth and the root image was taken by a root scanner and subsequent analysis was made by using the winRHIZO 2008a software.

		Means square				
Source of variation	**Df**	**Root length (cm)**	**Surface area (cm^2)**	**Root volume (cm^3)**	**Root tips**	**Average diameter (mm)**
Drought						
Lines	8	12013.8	189.20	0.022	101629.6	0.0214
Error	168	5115.7	73.34	0.0079	47978	0.0135
Total	176					
P-value		0.0229	0.0111	0.0062	0.0366	0.1348
Significance		*	*	**	*	ns
Well watered						
Lines	8	10111.23	115.36	0.009	63570.5	0.03203
Error	145	8292.2	107.20	0.0099	76150.8	0.0161
Total	153					
P-value		0.2917	0.383	0.518	0.5734	0.0512
Significance		ns	ns	ns	ns	ns

*and **indicates significance at $P<0.05$ and 0.01 respectively, ns= non-significant ($P>0.05$), Df = degree of freedom.

Appendix 5: Analysis of variance for the root architectural traits of nine bean lines under well watered and drought conditions for 4 weeks. The data represents six individual plants per plot (18 plants per bean lines) for each water regime.

Water regime	**Root architecture parameters**										
	Whorl			**Tap root**		**Basal root**			**Adventurous root**		
	No. of Whorl	1st Whorl angle	2nd Whorl angle	Width (mm)	Branching density	Number	Width (mm)	Branching density	Number	Width (mm)	Branching density
Well-watered											
Means square	0.364	557.49	451.24	2.111	29.22	6.55	0.339	35.06	52.07	0.39	35.23
P-value	0.055	0.227	0.0239	0.063	0.095	0.012	0.11	0.005	0.021	0.031	0.034
Significance	ns	*	*	ns	ns	*	ns	*	*	*	*
Drought											
Means square	0.186	482.68	379.76	1.079	40.06	6.0	0.39	27.65	0.349	1.079	47.46
P-value	0.426	0.006	0.047	0.195	0.001	0.043	0.196	0.0395	0.001	0.195	<0.0001
Significance	ns	**	*	ns	**	*	ns	*	ns	**	**

*and **indicates significance at $P \leq 0.05$ and 0.01 respectively, ns= non-significant

Appendix 6: Two ways ANOVA for nine common bean lines for biomass at flowering (Fl) and mid pod filling (MPF) stage for experimental variation of: lines (L), water treatment (W) and their interaction (L*W) for plants grown under field condition. The result represents for the plants exposed after 4 weeks drought stress.

		Means square					**Means square**
Variation	**d.f.**	**Total shoot dry mass at Fl (g)**	**Leaf dry mass at MPF (g)**	**Pod dry mass at MPF(g)**	**Total Shoot dry mass at MPF (g)**	**d.f.**	**Seed yield (kg/ha)**
Lines (L)	8	17.53 **	34.69 **	26.06 **	133.16 **	8	1468078 **
Water (W)	1	127.62 **	209.25 **	76.54 **	614.71 **	1	8523571 **
L*W	8	7.91 **	6.62 *	4.19 *	25.80 *	8	160518 *
Exp. error	90					36	
Total d.f.	107					53	

*and **indicates significance at P<0.05 and 0.01 respectively, d.f. = degree of freedom, Exp. = Experimental

Appendix 7: Analysis of variance for nine common bean lines for shoot and seed CID and C% as well as shoot C:N ratio under well-watered and drought conditions. Shoot samples were harvested after one month of drought exposure, at 30 days after planting.

Source of Variation	**DF**	**Shoot** CID	**Seed** CID	**Shoot C%**	**Seed C%**	**Shoot C:N ratio**
Well-watered						
Mean square						
Lines	8	0.533	3.8	4.729	1.187	49.230
Error	18	0.196	0.921	2.362	0.140	11.652
Total	26					
P-value		0.037	0.006	0.106	<.0001	0.005
Significance		*	**	ns	**	**
Drought						
Mean square						
Lines	8	0.421	0.918	1.3778	0.4964	56.467
Error	18	0.090	0.348	1.814	0.177	5.289
Total	26					
P-value		0.003	0.042	0.641	0.033	<.0001
Significance		**	*	ns	*	**

*and **indicate significance at P ≤ 0.05 and 0.01 respectively, ns= non significant, DF= degree of freedom

Appendix 8: Analysis of variance of seven common bean lines for shoot and seed $\delta^{15}N$ and %N as well as for calculated nitrogen derived from the atmosphere (Ndfa), plant N and fixed N (estimated from shoot %N,) (from 2.25 m^2 area) under well-watered and drought conditions. Shoot samples were harvested after one month of drought exposure, 30 days after planting.

Source of Variation	**DF**	**Shoot $\delta^{15}N$**	**Seed $\delta^{15}N$**	**Shoot %N**	**Seed %N**	**Ndfa**	**Plant N**	**Fixed N**
Well-watered								
Means square								
Lines	8	3.2471	5.6897	1.079	1.441	0.90	8.031	4.389
Error	18	1.083	0.240	0.202	0.117	0.982	1.023	1.257
Total	26							
P-value		0.025	<.0001	0.002	<.0001	0.511	0.002	0.025
Significance		*	**	**	**	ns	**	*
Drought								
Means square								
Lines	8	4.024	2.344	0.488	0.759	1.647	5.251	4.713
Error	18	0.388	0.80	0.071	0.219	0.460	0.238	0.214
Total	26							
P-value		<.0001	0.028	0.0003	0.014	0.023	<.0001	<.0001
Significance		**	*	**	*	*	**	**

*and **indicate significance at $P < 0.05$ and 0.01 respectively, ns= non-significant, DF= degree of freedom.

Appendix 9: Two way analysis of variance for for three soybean cultivars for root morphology traits for plants grown under well-watered and drought conditions. The data was obtained from root image taken by a root scanner and analysis made by using the winRHIZO 2008a software after 4 weeks of drought stress exposure.

Variation	**d.f.**	**Means square**			
		Root length (cm)	**Root surface area (cm^2)**	**Root volume (cm^3)**	**Root tips**
Cultivar (C)	2	13781.36 *	195.04 *	0.0262 *	89634.2 ns
Water (W)	1	22935.14 *	226.54 ns	0.0115 ns	327445.2 **
C*W	2	17227.88 *	380.03 **	0.0535 **	108768.0 *
Exp. error	116				
Total d.f.	121				

*and **indicates significance at $P<0.05$ and 0.01 respectively, ns = non-significant, d.f. = degree of freedom, Exp. = Experimental

Appendix 10: Two way analysis of variance for three soybean cultivars for biomasses at flowering (Fl) as well as mid pod filling stage (MPF) and seed yield for plants grown under field conditions in two water regimes (well-watered and drought conditions) after 4 weeks of drought stress exposure.

Variation	d.f.	Means square							
		Leaf dry mass at Fl (g)	**Stem dry mass at Fl (g)**	**Total dry mass at Fl (g)**	**Leaf dry mass at MPF(g)**	**Stem dry mass at MPF (g)**	**Pod dry mass at MPF (g)**	**Total dry mass at MPF (g)**	**Seed yield (kg/ha)**
Cultivar (C)	2	10.33 **	4.98 **	29.26 **	108.73 **	58.74 **	22.61 **	491.01 **	3590276 **
Water (W)	1	35.56 **	20.69 **	110.50 **	83.33 **	111.37 **	83.30 **	829.92 **	10666164 **
C*W	2	1.17 *	0.018 ns	1.17 ns	8.74 *	5.56 ns	14.62 **	57.67 *	1554792 **
Exp. error	116								
Total d.f.	121								

*and **indicates significance at P<0.05 and 0.01 respectively, ns = non-significant, d.f. = degree of freedom, Exp. = Experimental