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%, $\delta^{15}N$, %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)

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

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):

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

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):

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

%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 $\delta^{15}N$ 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 $\delta^{15}N$		Seed $\delta^{15}N$		Shoot %N	
	Well-watered	Drought	Well-watered	Drought	Well-watered	Drought
N-fixing						
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
Non fixing						
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.

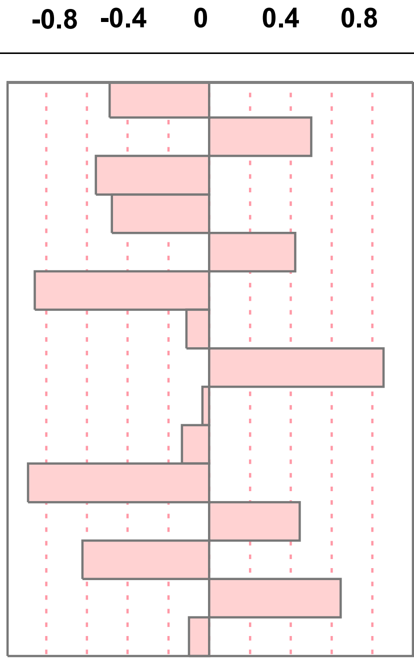
Lines	Seed %N		%Ndfa	Fixed N / 2.25 (g/m^2)	
	Well-watered	Drought	Drought	Well-watered	Drought
N-fixing					
BT_6-1-1	4.30±0.28a	4.29±0.36ab	57.54±2.2a	3.070±0.52ab	0.625±0.13c
BT_34-1-1	4.31±0.32a	4.55±0.37a	54.67±5.9ab	2.824±0.52bc	1.661±0.08b
BT_51-1-1	4.21±0.22a	3.64±0.21bc	47.46±2.7abc	1.836±0.06bc	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 $\delta^{15}N$ (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 $\delta^{15}N$, % N and from the computation of 2.25 m^2 for fixed N, and % Ndfa for plants grown under well-watered growth conditions.

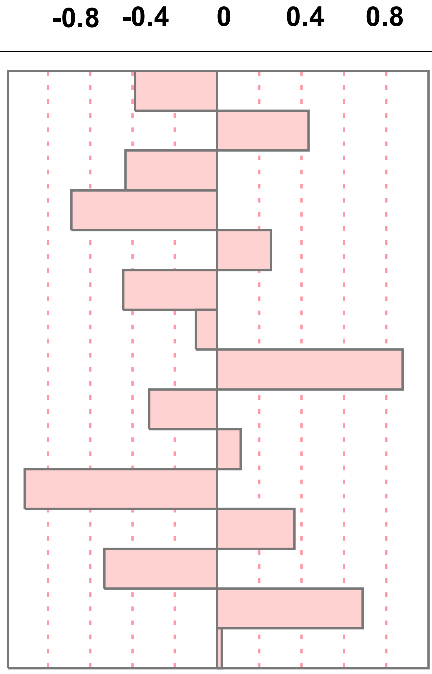
Traits		r-value	P-value
Shoot N%	Shoot d15N/14N	-0.4921	**
Seed 15N/14N	Shoot d15N/14N	0.5077	**
Seed 15N/14N	Shoot N%	-0.5635	**
Seed %N	Shoot d15N/14N	-0.4778	*
Seed %N	Shoot N%	0.4255	*
Seed %N	Seed 15N/14N	-0.8657	**
Fixed N/ plot	Shoot d15N/14N	-0.1156	ns
Fixed N/ plot	Shoot N%	0.8597	**
Fixed N/ 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 δ^{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 δ^{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 δ^{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 δ^{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 δ^{15}N was significantly ($P<0.05$), positively related with both shoot and seed CID except for shoot CID with shoot δ^{15}N and seed δ^{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 δ^{15}N with productivity parameters (seed yield and pod harvest index) was determined, the relation between δ^{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 δ^{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	0.548	0.475	0.548
	Ns	*	**	**
Shoot% N	-0.357	-0.627	-0.556	-0.230
	**	**	**	ns
Seed δ15N	0.583	0.388	0.700	0.527
	**	ns	**	*
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 $\delta^{15}N$ 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 $\delta^{15}N$	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 $\delta^{13}C$	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.

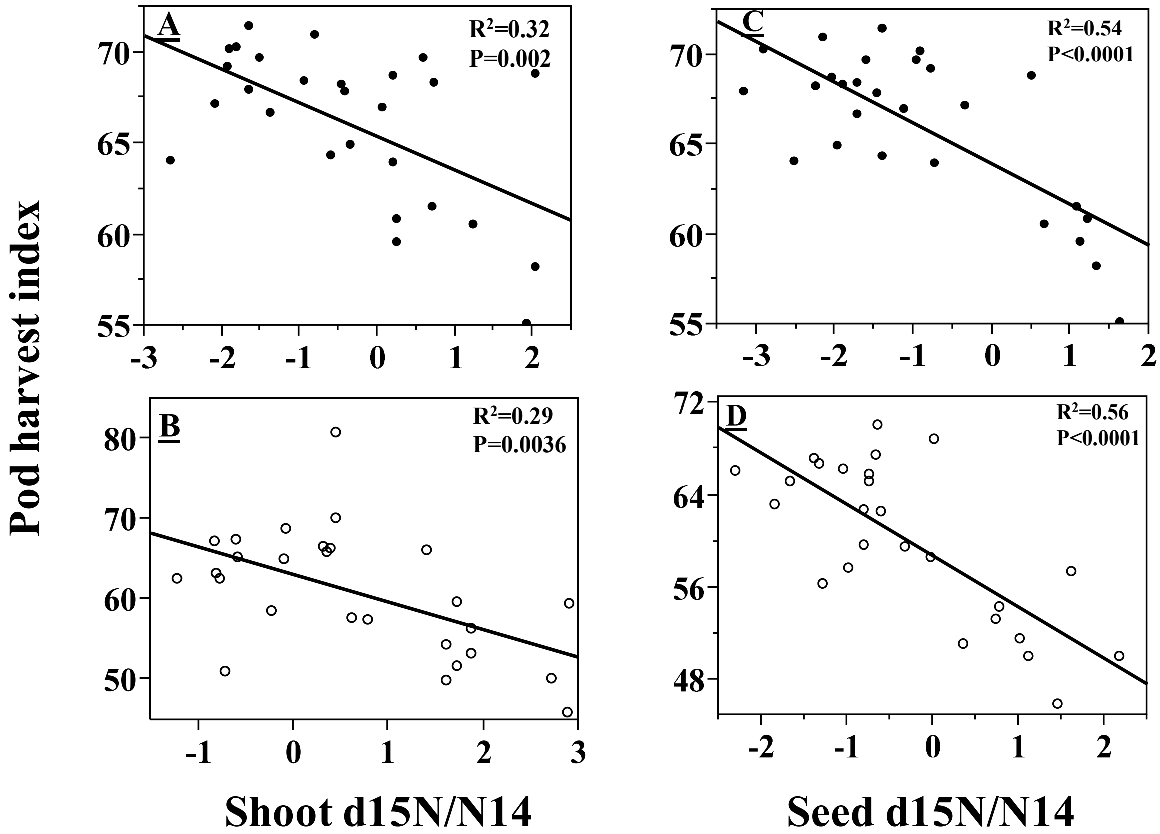


Figure 5.1 Relationship between shoot (A and B) and seed (C and D) d15N/14N (δ15N) and pod harvest index (PHI) of nine common bean lines. Data were obtained from one row per plot at harvest for PHI and analysis for $\delta^{15}N$ shoot and seed sample from plants grown under well-watered (A and C) and 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.

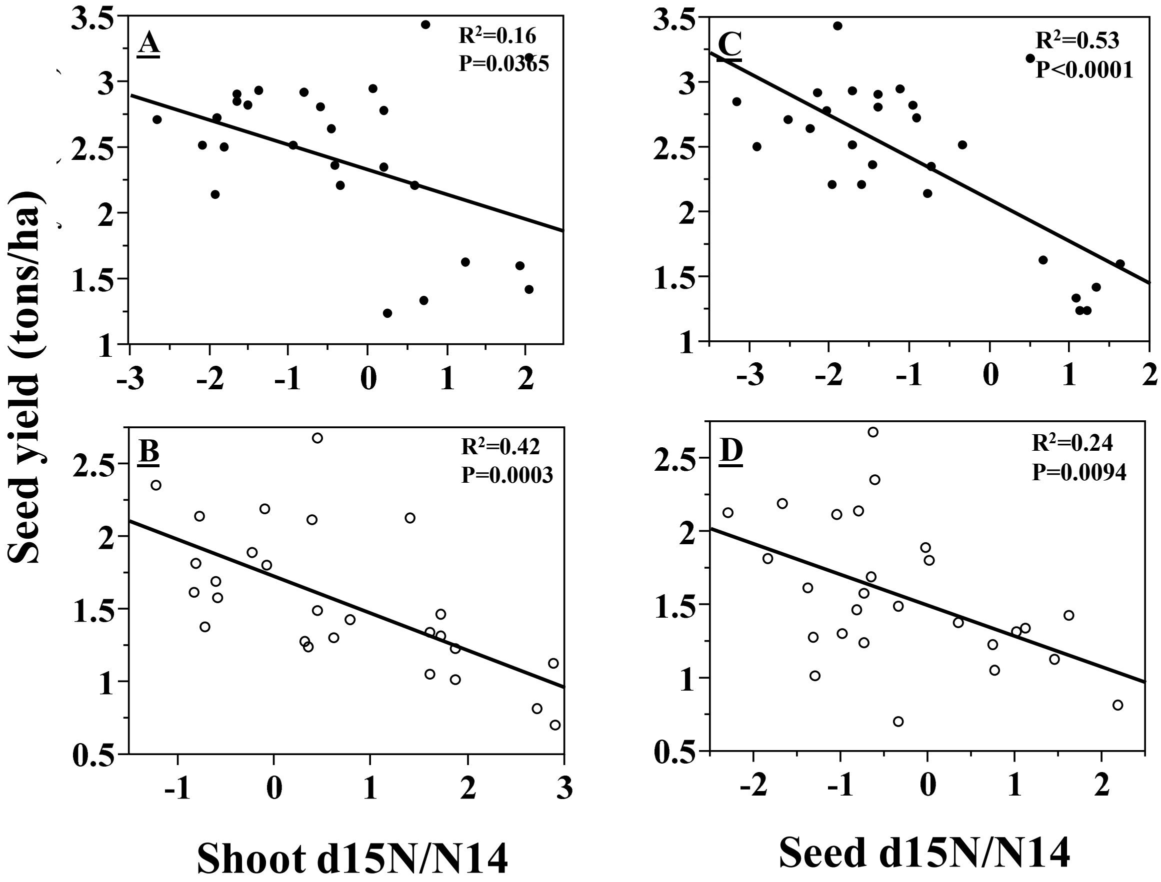


Figure 5.2 Association between shoot (A and B) and seed (C and D) $\delta^{15}N$ and seed yield of nine common bean lines. Data of seed yield were obtained from the harvest of two rows per plot and analysis for $\delta^{15}N$ seed sample per plot from plants grown under well-watered (A and C) and water-limited (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.