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ESTIMATION OF N_2-FIXATION OF MUNG BEAN AND MASH BEAN THROUGH XYLEM UREIDE TECHNIQUE UNDER RAINFED CONDITIONS

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Abstract

Xylem ureide is cost effective and inexpensive technique used for estimation of N_2-fixation in legumes. Legumes transport large amount of nitrogenous compound in the form of ureide. To quantify N_2-fixation through xylem ureide technique, field experiments were conducted on mungbean *(Vigna radiata)* and mashbean *(Vigna mungo)* during summer seasons of 2002 and 2003 at two different locations of Pothwar area i.e., Research Farm of University of Arid Agriculture, Rawalpindi (UAAR) and farmer's field in Chakwal district. Treatments were T_1) Mungbean, T_2) Mashbean, T_3) Mungbean + P @ 80 kg ha^{-1}, T_4) Mashbean + P @ 80 kg ha^{-1}. Bean seeds were inoculated at sowing with effective brady rhizobia. Relative abundance of ureide-N (100×4×ureide/(4×ureide + nitrate + α amino-N) has been used as an indicator for relative dependence on N_2-fixation. Concentration of ureide, nitrate and α amino-N were determined at pod filling stage to calculate the relative abundance of ureide-N (RUN%) and proportion of plant N derived from N_2-fixation (%P_{fix}). Highest shoot dry matter (DM) yield 4.0 t ha^{-1} (85 kg N ha^{-1}) of mash bean was recorded at UAAR site during summer 2003. Mung and mash beans fertilized with phosphorus produced 24 and 28% higher nodules when compared to beans without fertilizer, respectively. Ureide concentration (0.69 mM) was maximum in the xylem sap of mash bean with phosphorus at Chakwal site. The relative abundance of uredide-N (RUN%) in the xylem sap of beans, was in the range of 36-60 %. An increase of 32 % in the value of %P_{fix} was observed by addition of phosphorus fertilizer. N_2-fixation by mung bean and mash bean was 47 and 80 kg N ha^{-1}, which tended to increase up to 24 % with application of phosphorus fertilizer. Regression analysis indicated close association of N_2-fixed with shoot DM (R^2 = 0.88) and shoot N (R^2 = 0.90) at UAAR site. Correlation amongst the parameters for the legumes showed that N_2-fixation was positively and strongly correlated with all the legume parameters i.e., shoot dry matter (r = 0.90), shoot N (r = 0.79) and %P_{fix}(r = 0.99) at Chakwal site.

Introduction

Availability of reduced nitrogen is an important determinant in the growth and development of plants. Although in most vascular plant species, the major transport form of reduced/organic nitrogen is as amino acids (including amides). Tropical and subtropical legumes like cowpea (*Vigna unguiculata*), soybean (*Glycine max*), and French bean (*Phaseolus vulgaris*) transport large amounts of the nitrogenous compounds called ureides (Sinclair & Serraj 1995). The dominant forms of ureides in these species are allantoin and allantoic acid (Pate *et al.,* 1980; Thararajah *et al*., 2005). In legumes that are adapted to temperate climates (e.g., pea [*Pisum sativum*] and faba bean [*Vicia faba*]), the amides glutamine (Gln) and asparagine (Asn) take on the major transport function (Herridge *et al.,* 1978; Schubert. 1986; Thararajah *et al*., 2005). Ureides can comprise up to 90% of the total nitrogen transported in the xylem of nitrogen-fixing tropical legumes (Herridge *et al.,* 1978; Pate *et al.,* 1980) and can be stored in high amounts in the different plant organs (Matsumoto *et al.,* 1977; Streeter, 1979; Layzell & LaRue, 1982).

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Nitrogen fixation cannot be assessed unless a reliable and accurate field measurement is made of the levels of fixation achieved. So, measurement of nitrogen fixation enables to evaluate (a) the ability of indigenous *Rhizobium* spp., to effectively nodulate newly introduced legume, (b) the symbiotic effectiveness of legume. Technically, the field sampling of xylem contents is simple and the analysis of N-components (i.e., ureide, nitrate and amino-N) can be done by colorimetric assays in a test tube. There is no need for expensive or sophisticated equipment and many analyses may be performed daily. It is not necessary to dig out legume roots and recover nodules to obtain measures of N_2-fixation, nor is it necessarily a totally destructive technique as sufficient sap can be collected from stem segment and laterals of mature plant for complete analysis (Herridge *et al.,* 1988). Since sampling is confined to the accessible aerial parts of the plants, the solute method may potentially overcome many problems associated with measuring N_2-fixation by twining ground cover or forage legumes (Norhayati *et al.,* 1988). In natural ^{15}N abundance method, it is necessary to have the non N_2-fixing plants to minimize the effect of any site variability on measurements. This technique also requires a precise mass spectrometer, meticulous analytical procedure and is also very expensive.

Ureide measurement can be readily and actively inexpensively used technique to evaluate N_2-fixation in most tropical legumes. This technique relies on the observation that in certain legumes, pathways of assimilation of N derived from fixation and from soil are different: ammonia derived from symbiotic fixation is converted into the Ureides, allantoin and allantonic acid, in the nodule and then transported to the shoot in the chemical form in the transpiration stream; in contrast, N taken up from soil, which is primarily nitrate is transported either directly as nitrate or is assimilated into the amino acids asparagines or glutamine in the root prior to transport (Herridge & People., 1990). Therefore xylem sap composition changes from one dominated by ureides in fully symbiotic plants to one dominated by nitrate and amino acids in poorly nodulated plants utilizing soil N for growth. The proportion of total sap N formed by Ureides has been shown to be a reliable indicator of the $\%P_{fix}$ for a number of legume species including mung bean and mash bean and comparable field estimates of N_2-fixation can be obtained using either Ureide or ^{15}N-based methodologies (Herridge *et al.,* 1990).

Functions relating legume N_2-fixation to crop growth and soil nitrate should be developed for use as management tools by farmers. Legume N_2-fixation is linked positively to yield. With accurate knowledge of effects of these factors on N_2-fixation, farmers ought to be able to increase inputs of N_2-fixed into cropping systems through improved management of the legumes phase of the rotation. Estimates of N_2-fixation and nitrogen (N) balances (i.e., fixed-N_2 minus grain N) of legume crop should allow farmers to assess the likely impact of the legume on the nitrate-N levels in the soil and eventually on the N status of a following cereal crop. The outcome would be improved management of N in the cropping system (Herridge *et al*., 1998).

Instead of very sophisticated and costly methods like ^{15}N-isotopic techniques, we used reliable and cost effective technique for measuring N_2-fixation of mung and mash beans with the objectives to: (i) observe the effect of inoculation and phosphorus fertilizer on nodulation, dry matter and N yield of mung and mash beans, (ii) determine the composition of N solute (ureide, nitrate and α amino-N) in the xylem sap of mung and mash beans, (iii) estimate $\%P_{fix}$ and N_2-fixed by mung and mash beans and (iv) establish functions relating legume N_2-fixation to crop growth under rainfed conditions.

Materials and Methods

Field experiments were conducted on mungbean *(Vigna radiata)* and mashbean *(Vigna mungo)* during summer seasons of 2002 and 2003 to quantify N_2-fixation through xylem ureide technique. The experiments were conducted at two different locations of Pothwar area i.e., Research Farm of University of Arid Agriculture, Rawalpindi (UAAR) and farmer's field in Chakwal district. The rainfall pattern of the experimental site is bimodal with summer dominance (70% between July and September). Rainfall data was collected at Regional Agro Meteorological Center (RAMC, UAAR & Chakwal) and is presented in Fig. 1. Mung (Var. NM-92) and mash beans (Var. Mash-3) were inoculated at sowing with effective brady rhizobia and were sown with seed rate 20 and 18 kg ha^{-1}, respectively. Rhizobium inoculant (10^9 - 10^{10} bacterial cell g^{-1} inoculants) was provided by Soil Biology and Biochemistry Section, National Agriculture Research Center (NARC), Islamabad and been seeds; inoculant and sugar were thoroughly mixed before sowing. The legumes were grown with and without Phosphorus fertilizer. Phosphorus was applied @ 0 and 80 kg ha^{-1} for both beans crop in the form of single super phosphate. Each experiment was replicated four times in a Randomized Complete Block Design. The net plot size was 5×5 m. Treatments were T_1) mungbean, T_2) mashbean, T_3) mungbean + P @ 80 kg ha^{-1}, T_4) mashbean + P @ 80 kg ha^{-1}.

Roots (five) were excavated from 1m^2 area randomly selected from each plot for nodulation. The nodule grade was determined on the basis of nodule number and mean nodule grade was determined by using visual rating criteria given by Rupela, (1990). At crop maturity, all plants in a 1m^2 quadrate in each plot were harvested, oven dried at 80 ^{o}C to a constant mass, then weighed. Shoot N concentrations were determined by colorimetric procedures (Anderson & Ingram, 1993).

N_2-fixation was estimated by Xylem Solute Technique (Peoples *et al.,* 1989). Sap was collected at the pod-fill stage by Vacuum Extraction Method and stored in the freezer at -15 ^{o}C, then concentrations of ureide, nitrate and amino-N were determined (Peoples *et al.,* 1989) to calculated the relative abundance of ureide (RUN%) and % P_{fix} (proportion of plant N derived from N_2-fixation) by the following formula:

$$\text{RUN (\%)} = [4 \times \text{ureide} / (4\times \text{ureide} + \text{nitrate} + \text{amino-N})] \times 100$$

After getting the value of RUN%, the proportion of plant N derived from N_2-fixation (%P_{fix}) was estimated.

$$\%P_{fix} = 1.6\ (\%\text{RUN}-15.9) \text{ for plants during pod fill}$$

The legume N was derived from the measure of biomass accumulation and tissue N-content as follows:

$$\text{Crop N (kg ha}^{-1}) = \text{Legume dry matter (kg ha}^{-1}) \times (\%\text{N})$$

The amount of nitrogen fixed by legume can be regulated by two factors, the amount of N accumulated during growth, and the production of that N derived from symbiotic N_2-fixation.

$$\text{Amount of } N_2\text{-fixed (kg ha}^{-1}) = \%P_{fix} \times \text{Crop N (kg ha}^{-1}) \times 1.5^*$$

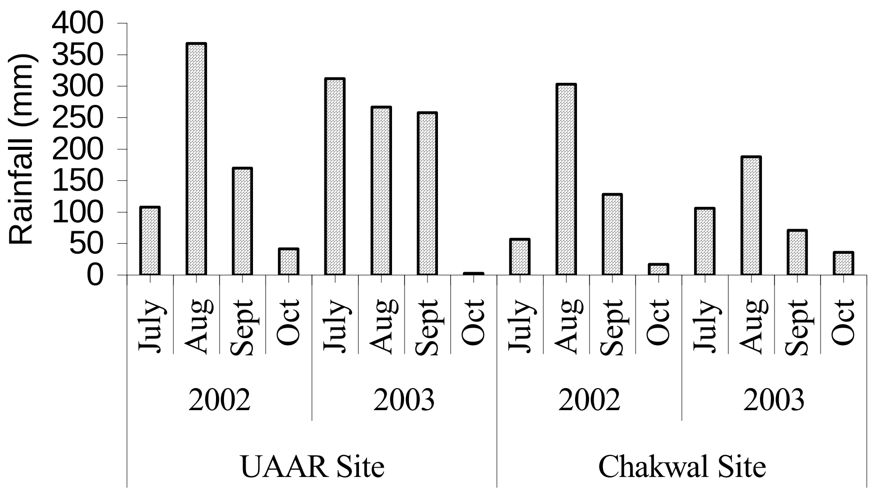

Fig. 1. Rainfall (mm) during experiments at both locations.

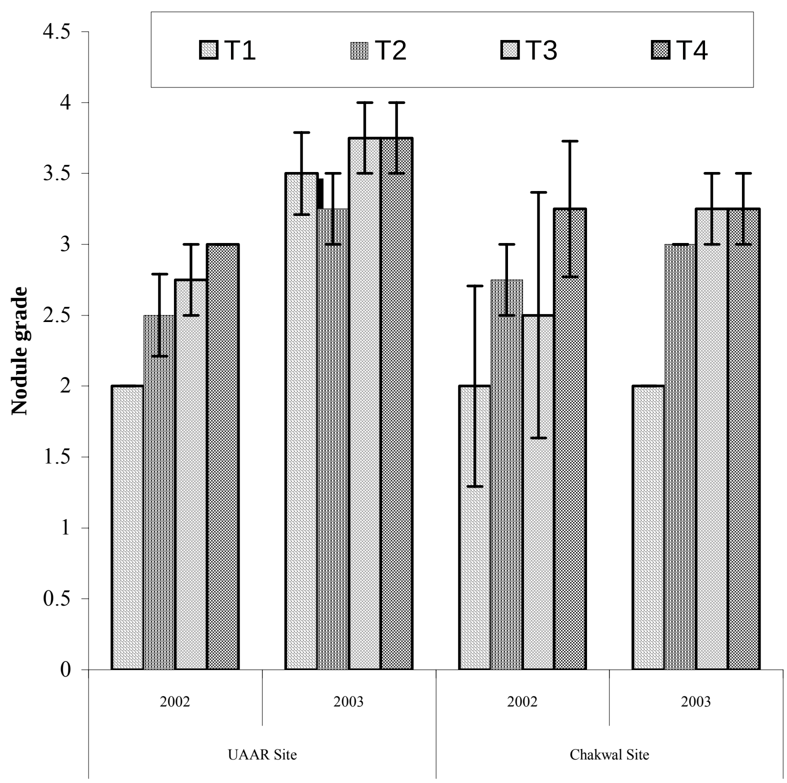

Fig. 2. Nodule grading of mung bean and mash bean.
T_1) Mung bean, T_2) Mash bean, T_3) Mung bean + P @80 kg ha^{-1}, T_4) Mash bean+ P @80 kg ha^{-1}

*1.5 factor was used to include an estimate for contribution by below ground N (Peoples *et al.,* 1989). The data collected for various characteristics were subjected to statistical analysis using Randomized Complete Block Design. Two years data of beans were combined for reliable results. A software package MStat C was used to calculate ANOVA Tables. Treatments means were separated by using DMRT at $p<0.05$.

Results

The data showed that inoculation along with phosphorus fertilizer had a significant effect on nodulation, shoot dry matter and shoot N yield in both beans (Table 1). During 1st year of experiment, both beans were less affected by applied phosphorus at both sites. However, during 2nd summer of 2003, shoot dry matter of mung bean showed 4 & 7% increase with phosphorus fertilization at UAAR and Chakwal site, respectively (Table 1). Highest Shoot DM yield (4.38 t ha^{-1}) and shoot N (85 kg N ha^{-1}) of mash bean were recorded at UAAR site during 2003. It was also observed that shoot dry matter yield of both beans were 37% higher at UAAR site as compared to Chakwal site. During 1st year, much better nodulation (i.e., nodule grade 3) was observed with phosphorus (Fig. 2), while 2nd year data showed nodule grade of 4, representing an excellent nodulation and equally excellent potential for nitrogen fixation at UAAR site. Nodule grade was statistically (24-28%) higher with phosphorus fertilization during summer 2003 (Fig. 2). At Chakwal site, maximum nodule grade of 3 was observed in beans with phosphorus fertilizers.

Concentration of ureide, nitrate and α amino-N (Table 2) were determined at pod filling stage to calculate the relative abundance of ureide-N (RUN%) and $\%P_{fix}$ (Table 3). During 1st year of experiments, no much differences of ureide concentration (range 0.41-0.50 mM) were found with and with out phosphorus fertilization. The maximum ureide concentration at UAAR site was 0.67 mM in mung bean at zero phosphorus level while 0.69 mM in mash bean at 80 kg P ha^{-1} at Chakwal site in 2003. Nitrate concentration was maximum in mung bean at zero phosphorus level in 2003 at both sites. Highest α amino-N concentration (2.09 mM) was observed in mung bean fertilized with phosphorus at Chakwal site in 2002. During 2003, α amino-N concentration (2.07 mM) was again higher in mung bean without phosphorus fertilization at same site. The relative abundance of ureide-N (RUN%) in the xylem sap of beans was in the range of 36-60 %. During 2002, phosphorus effect on RUN% was negligible at both sites. However, during 2003, RUN% values were significantly higher (21%) in mash bean at 80 kg P ha^{-1} then mash bean with zero phosphorus at UAAR site. RUN% value in mash bean with phosphorus fertilizer was 8% higher then mash bean with zero phosphorus at Chakwal site in 2003.

At UAAR Site, during summer 2002, under normal soil fertility condition, value of P_{fix} was 40% by mung bean which tend to increase up to 5% with addition of phosphorus. Similarly, P_{fix} by mash bean was 41% without phosphorus and 6% increase in $\%P_{fix}$ was noted by addition of phosphorus fertilizer. Maximum P_{fix} (44%), occurred in treatment with mash bean under phosphorus fertilization which was 3% higher when compared to same treatment of mung bean. In the second year, during summer 2003, under normal soil fertility condition, value of P_{fix} was 49% in mung bean which tended to increase up to 15% with addition of phosphorus fertilization. Similarly, P_{fix} by mash bean was 54% without phosphorus and 32% increase in $\%P_{fix}$ was observed by addition of phosphorus fertilizer. Average value of P_{fix} for both years at UAAR site was 57% & 50% in mash bean and mung bean fertilizes with phosphorus and $\%P_{fix}$ value was 20 and 11% higher in mash bean and mung bean with phosphorus fertilizer then mash bean and mung bean without phosphorus, respectively. At Chakwal site, maximum P_{fix}, 38%, occurred in treatment with mash bean under phosphorus fertilization which was 15% higher when compared to that of mung bean without phosphorus.

Table 1. Shoot dry matter and N yield of beans.

Crops	P level ($kg\ ha^{-1}$)	Shoot DM ($T\ ha^{-1}$)	Shoot N %	N uptake[A] ($kg\ ha^{-1}$)	Shoot DM ($T\ ha^{-1}$)	Shoot N %	N uptake[A] ($kg\ ha^{-1}$)
		UAAR Site					
		Summer 2002			**Summer 2003**		
Mung bean	0	1.70 (0.11) cd	1.81 (0.23) ab	30.79 (2.32) gh	3.88 (0.28) a	1.63 (0.06) b-e	63.42 (5.34) c
Mash bean	0	1.18 (0.04) d	1.80 (0.16) a-c	21.24 (1.64) hi	4.27 (0.21) a	1.99 (0.33) a	84.85 (5.14) a
Mung bean	80	1.80 (0.17) c	1.45 (0.10) de	26.25 (3.44) hi	4.18 (0.22) a	1.65 (0.06) b-e	68.51 (2.65) bc
Mash bean	80	1.19 (0.13) d	1.61 (0.06) b-e	19.15 (2.59) i	4.38 (0.21) a	1.72 (0.07) a-d	75.30 (3.36) ab
		Chakwal Site					
Mung bean	0	2.76 (0.41) b	1.37 (0.06) e	37.80 (5.31) fg	3.05 (0.40) b	1.53 (0.06) b-e	46.36 (5.59) d-f
Mash bean	0	2.82 (0.26) b	1.52 (0.05) b-e	42.82 (2.67) d-f	3.08 (0.19) b	1.59 (0.13) b-e	48.99 (4.90) de
Mung bean	80	2.87 (0.28) b	1.39 (0.05) de	39.91 (3.43) e-g	3.25 (0.22) b	1.51 (0.14) c-e	49.20 (6.20) de
Mash bean	80	3.02 (0.11) b	1.54 (0.03) b-e	46.51 (2.04) d-f	3.20 (0.13) b	1.63 (0.09) b-e	52.37 (5.01) d
LSD ($p<0.05$)	0.58	0.09	9.77	0.58	0.09	9.77	

Data in the parenthesis show SDEV; [A](shoot dry matter $kg\ ha^{-1}$) × (shoot %N)

Means followed by the same letter (s) are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT).

Data is average of 4 replicates.

Table 2. Ureide, Nitrate and Amino N contents of xylem sap.

Crops	P level (kg ha^{-1})	Ureide	Nitrate	Amino-N	Ureide	Nitrate	Amino-N
		mM			mM		
		UAAR Site					
		Summer 2002			Summer 2003		
Mung bean	0	0.50 (0.07) a-c	1.05 (0.17)*	1.85 (0.11) a-d	0.67 (0.12) a	1.25 (0.23)*	1.83 (0.36) a-d
Mash bean	0	0.42 (0.04) bc	0.67 (0.15)	1.67 (0.07) a-d	0.53 (0.12) a-c	1.01 (0.25)	1.15 (0.26) ef
Mung bean	80	0.46 (0.03) bc	0.96 (0.16)	1.53 (0.09) c-e	0.54 (0.06) a-c	0.60 (0.05)	1.43 (0.14) de
Mash bean	80	0.44 (0.08) bc	0.78 (0.23)	1.54 (0.2) b-e	0.49 (0.02) a-c	0.48 (0.12)	0.83 (0.01) f
		Chakwal Site					
Mung bean	0	0.40 (0.02) c	1.02 (0.15)*	1.83 (0.11) a-d	0.62 (0.04) ab	1.27 (0.21)*	2.05 (0.04) ab
Mash bean	0	0.41 (0.08) bc	0.93 (0.28)	1.76 (0.25) a-d	0.54 (0.05) a-c	1.07 (0.11)	1.78 (0.09) a-d
Mung bean	80	0.41 (0.02) bc	0.78 (0.11)	2.09 (0.12) a	0.61 (0.04) a-c	1.07 (0.11)	1.94 (0.07) a-d
Mash bean	80	0.49 (0.05) a-c	1.23 (0.25)	1.76 (0.18) a-d	0.69 (0.04) a	1.23 (0.09)	1.98 (0.05) a-c
LSD ($p<0.05$)	0.58	0.18	1.43	0.44	0.18	1.43	0.44

Data in the parenthesis show SDEV; Means followed by the same letter (s) are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT). Data is average of 4 replicates; *Non- significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT).

Table 3. N_2-fixation of mung baen and mash bean.

Crops	P level (kg ha^{-1})	RUN^A %	P_{fix}^{B} %	N_2-Fixed^C (kg ha^{-1})	RUN^A %	P_{fix}^{B} %	N_2-Fixed^C (kg ha^{-1})
		UAAR Site					
		Summer 2002			**Summer 2003**		
Mung bean	0	41.08 (5.14) c-e	40.28 (8.23) c-e	18.86 (4.41) h-j	46.62 (0.97) b-d	49.15 (1.55) b-d	46.58 (3.45) d
Mash bean	0	41.71 (4.05) c-e	41.30 (6.47) c-e	13.33 (2.77) j	49.54 (1.23) bc	53.82 (1.96) bc	68.31 (3.77) b
Mung bean	80	42.46 (0.80) b-e	42.49 (1.28) b-e	16.59 (1.85) ij	51.33 (2.53) b	56.70 (4.01) b	57.93 (3.06) c
Mash bean	80	43.19 (5.95) b-e	43.67 (9.52) b-e	12.90 (3.57) j	60.21 (2.25) a	70.90 (3.60) a	79.72 (3.12) a
		Chakwal Site					
Mung bean	0	36.04 (1.39) e	32.23 (2.23) e	18.54 (3.29) h-j	42.77 (0.78) b-e	42.99 (1.26) b-e	29.63 (3.25) e-h
Mash bean	0	37.92 (0.96) de	35.23 (1.54) de	22.54 (1.30) g-j	42.79 (2.02) b-e	43.03 (3.24) b-e	32.25 (5.52) e-g
Mung bean	80	36.37 (0.46) e	32.75 (0.73) e	19.71 (2.11) h-j	44.86 (2.84) b-e	46.33 (4.55) b-e	35.43 (7.91) ef
Mash bean	80	39.38 (3.00) de	37.57 (4.81) de	26.08 (3.36) f-i	46.31 (1.96) b-d	48.66 (3.14) b-d	38.26 (4.35) de
LSD ($p<0.05$)	0.58	7.88	12.62	10.03	7.88	12.62	10.03

^{A}(4 × ureide/4×ureide + nitrate + amino-N) × 100 ; ^{B}(106 × (%RUN – 15.9) ; ^{C}(%*Pfix* at pod fill stage) × (shoot N kg ha^{-1}) × 1.5

Mean followed by the same letter (s) are not significantly different ($P<0.05$) according to Duncan's Multiple Range Test (DMRT).

Data is average of 4 replicates.

Data in the parenthesis showed SDEV

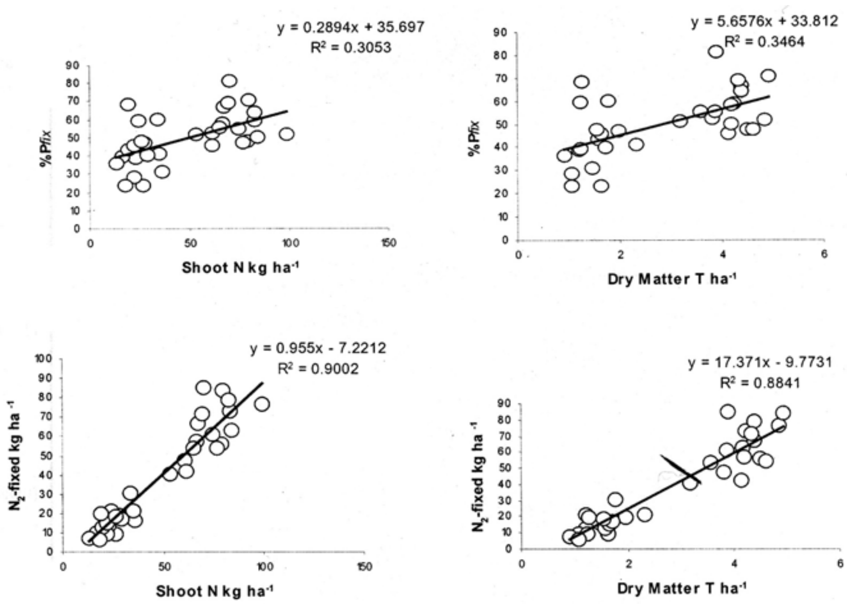

Fig 3. Relationship between Shoot DM & Shoot N yield with proportion of plant-N derived from N_2-fixation (%P_{fix}) and N_2-fixation of beans at UAAR Site.

Table 4. Correlation matrix of shoot DM, N yield and N_2-fixation of beans.

	Shoot DM	Shoot N	N Uptake	Ureide	Nitrate	Amino-N	RUN	P_{fix}
				UAAR Site				
Shoot N	-0.88							
N Uptake	-0.54	0.83						
Ureide	0.07	-0.03	-0.40					
Nitrate	-0.14	0.29	-0.06	0.94				
Amino-N	0.08	0.09	-0.19	0.96	0.97			
RUN	0.02	-0.37	-0.26	-0.72	-0.89	-0.88		
P_{fix}	0.02	-0.37	-0.26	-0.72	-0.89	-0.88	1.00	
N_2-Fixed	-0.29	0.04	0.19	-0.90	-0.90	-0.97	0.89	0.89
				Chakwal Site				
Shoot N	0.44							
N Uptake	0.80	0.89						
Ureide	0.72	0.44	0.67					
Nitrate	0.11	0.45	0.37	0.75				
Amino-N	0.17	-0.79	-0.43	0.14	-0.22			
RUN	0.89	0.75	0.95	0.84	0.47	-0.18		
P_{fix}	0.89	0.75	0.96	0.84	0.47	-0.18	1.00	
N_2-Fixed	0.90	0.79	0.98	0.74	0.35	-0.25	0.99	0.99

The data on N_2-fixation revealed that both mung bean and mash bean fixed substantial but variable quantities of nitrogen per hectare (Table 3). At UAAR Site, during summer 2002, no variations were recorded among different treatments. However, during summer 2003, under normal soil fertility condition, N_2-fixation by mung bean was 47 kg ha^{-1} which tended to increase up to 24 % with application of phosphorus fertilizer. Value of N_2-fixed by mash bean was increased up to 17% with phosphorus fertilization in 2003 at UAAR site. Similarly at Chakwal site, value of N_2-fixed by both beans was 19%

higher with phosphorus fertilization as compared to beans receiving zero phosphorus in 2003. Data were also subjected to simple regression analysis with $\%P_{fix}$ and N_2-fixation as the dependent variables and shoot DM and shoot N yield as independent variables (Fig. 3). Simple regression functions for $\%P_{fix}$ with shoot DM and N yield was not strongly correlated at both sites. However, N_2-fixation was more strongly correlated with shoot N (R^2 = 0.90) and shoot DM (R^2 = 0.88) at UAAR site. Correlation amongst the parameters for the legumes are presented in Table 4. N_2-fixation was negatively correlated with shoot dry matter and N solutes (ureide, nitrate and α amino-N) in xylem sap and positively correlated with shoot N and $\%P_{fix}$ at UAAR site. N_2-fixation was positively and very high correlated with all the legume parameters i.e., with shoot dry matter (r = 0.90), with shoot N (r = 0.79), and with $\%P_{fix}$(r = 0.99) at Chakwal site.

Discussion

Plant growth (DM yield), symbiotic parameters (nodule number, nodule grade) and N solutes (Ureide, amino-N and nitrate) in xylem sap, all values were observed higher during 2^{nd} summer of 2003 indicating integrated residual effect of legumes and phosphorus fertilization and favorable environment supports, good cropping stand and N yield. Mung bean can be successfully grown under limited water supply with phosphorus fertilizer (Malik *et al*., 2006). NPK fertilizers and inoculation with bradyrhizobium enhanced nodulation; shoot biomass and grain yield of mash bean (Javid *et al*., 2006). Phosphorus along with rhizobium inoculation increased growth, yield and nitrogenase activity as well as improved soil fertility for sustainable agriculture (Gentili & Huss-Danell, 2003; Fatima *et al*., 2007). By comparing two sites it was observed that at UAAR, RUN values (47%) were 15% higher when compared with Chakwal Site (41%). 21 % increase was observed during summer 2003 as compared to summer 2002. Significantly higher $\%P_{fix}$ value was observed (25% increase) at UAAR site when compared to Chakwal site. Similarly, 35% higher P_{fix} values were observed during summer 2003 over summer 2002. Increment in the value of P_{fix} was 9% with the addition of phosphorus fertilization at Chakwal site. These results are in the line with those of Ali *et al.,* (1999) who reported that the P_{fix} values for soybean, mung bean and mash bean were 16, 31 and 46% in 1996 (1^{st} year) and 31-37 and 48% during 2^{nd} year of 1997. Experimental treatments and environmental or nutritional variable have generated a large range of P_{fix} values, 0- 98% (Peoples *et al.,* 1995). The $\%P_{fix}$ data were also similar to those reported by Shah *et al.,* (1997) and Maskey *et al*., (2001) from farmer crop survey's of the same species in North West Frontier Province of Pakistan (mean of 47%) and Hilly and Terai regions of Nepal (mash bean mean of 47%), respectively.

The data on N_2-fixation (Table 3) revealed that both mung bean and mash bean fixed substantial but variable quantities of nitrogen per hectare. N_2-fixation value was observed highest (80 kg N ha^{-1}) in mash bean with 80 kg P ha^{-1} at UAAR site in 2003. The mean of two years on N_2-fixation by mung bean and mash bean at Chakwal site, indicated that the mung bean with 80 kg P ha^{-1} fixed 27kg N ha^{-1}, 12% higher than that of mung bean with zero phosphorus. N_2-fixation value (32 kg N ha^{-1}) in mash bean with 80 kg P ha^{-1} was also 18% higher than mash bean with zero phosphorus. Similar result were also observed in comparison between mung bean and mash bean (Ahmad *et al.,* 2001) that mash bean varieties were slightly better N fixer than the mung bean varieties. Significant difference was observed between the two sites with respect to N_2-fixation; more N_2-fixation was observed at UAAR site than Chakwal site. With a favorable summer rains and ideal growth conditions at the UAAR site in 2003, mash bean with phosphorus fertilization fixed 80 kg N ha^{-1} roughly twice that recorded at the farmer's fields in Chakwal during the same year.

Similar high values have also been reported in good rainfall years for forage legumes in research trials in the Kathmandu valley, Nepal (Schulz *et al.,* 1999). Inoculation with suitable rhizobia along with Phosphorus improves symbiotic nitrogen fixation and yield in common bean (Zaman-Allah *et al*., 2006) and soy bean (Fatima *et al*., 2007).

Values of Regression coefficients (R^2) showed that N_2-fixation was strongly correlated with shoot DM and N yield at UAAR site (Fig. 3), However, the regression for both P_{fix} and N_2-fixation at Chakwal site was not strong because of variation in the relationship between shoot DM and N yield associated with growth and environmental effects as well as all the difficulties in sampling at farmer's field conditions. Plotting the relationship between shoot N and shoot N fixed has proved useful in other studies for comparing and contrasting legume N_2-fixation in different system. Such an approach also highlighted differences in the factors regulating N_2-fixation in Australia (Peoples *et al.,* 2001). The use of shoot dry matter and N yield as independent variable is particularly important. A substantial research work showed that plant (crop) yield is the major determinant of N_2-fixation, particularly when the levels of nitrate in the soil are low, and effective rhizobia are present in the soil in sufficient number (Evans *et al.*, 1989; Herridge *et al.*, 1998). Yield in these instances is often related to species or cultivar, mediated through growth rates or crop duration, and to nutrition and water availability. Positive correlation showed that total N_2-fixed by beans was influenced primarily by crop growth i.e., shoot DM and N (Table 4, Fig. 3). The differences in crop growth and N_2-fixation may be partially explained by differences in year to year variations in rainfall. Field observation also indicated particular problems with plant populations, insect and disease damage and weed incidence in specific crops which undoubtedly limited growth potential.

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