

## BIOLOGICAL NITROGEN FIXATION OF SUMMER LEGUMES AND THEIR RESIDUAL EFFECTS ON SUBSEQUENT RAINFED WHEAT YIELD

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### Abstract

Biological nitrogen fixation is the most important biochemical reaction for life on earth. Phosphorus and rhizobium inoculation increased N<sub>2</sub>-fixation by legumes. Legumes in rotation with cereals contribute to the total N pool in soil and improved cereals yield. In view of importance of grain legumes and the role they can play in maintaining soil productivity and succeeding cereal yield, rotational field experiments were conducted on mung bean (*Vigna radiata*) and mash bean (*Vigna mungo*) during summer of 2002 and 2003 followed by wheat (*Triticum aestivum*) in each year at Research Farm of University of Arid Agriculture, Rawalpindi, to assess N<sub>2</sub>-fixation by beans and their residual effect on subsequent wheat yield. Bean seeds were inoculated at sowing with effective brady rhizobia and grown with and without Phosphorus fertilizer. Sorghum (Var.YSS-98) was sown as non-legume crop with 100kg N ha<sup>-1</sup>. Xylem ureide method has been employed for estimation of N<sub>2</sub>-fixation. Nodulation, shoot dry matter, grain yield and N concentration of both beans were increased by phosphorus fertilization. Both beans showed excellent nodulations i.e., 4, which showed excellent potential for nitrogen fixation. Estimates of nitrogen derived from atmosphere (%P<sub>fix</sub>) ranged from 49-71% during 2<sup>nd</sup> year and up to 60% increase was observed from 1<sup>st</sup> year. Average N<sub>2</sub>-fixed ranged between 33-55 kg ha<sup>-1</sup> during both years and mash bean proved better N<sub>2</sub>-fixer. Water use efficiency (WUE) based on grain yield were 23-33% higher with phosphorus fertilization. Values of WUE for N<sub>2</sub>-fixation ranged between 0.22 and 1.00 kg ha<sup>-1</sup> mm<sup>-1</sup> and declined with declining %P<sub>fix</sub>. Total NO<sub>3</sub>-N was between 56-67 kg ha<sup>-1</sup> for legumes and between 40-45 kg ha<sup>-1</sup> for non-legume sorghum. Additional residual soil N under legumes, relative to adjacent sorghum was in the range of 16-22 kg ha<sup>-1</sup>. Beans with phosphorus fertilization increased grain yield of succeeding wheat by 20% over sorghum. It was concluded that phosphorus fertilizer with inoculation enhanced N<sub>2</sub>-fixation and rotational results confirmed that legume-cereal sequence increased biomass and grain yield of subsequent wheat.

### Introduction

The use of nitrogenous fertilizers in Pakistan is limited because of high cost, poor economic conditions of the farmers, inadequate credit facilities and their non-availability at proper time. This necessitates the inclusion of leguminous crops in our cropping systems as these have the ability of enriching the nitrogen content of the soil by fixing nitrogen from the air, in addition to improving the productivity of soil. Cereals, lacking symbiotic arrangements, require soil nitrogen or fertilizer for satisfactory growth. It has been reported that the net benefits of legumes are often equivalent to the addition of 50-100 kg N ha<sup>-1</sup> as fertilizer (Herridge *et al.*, 1993; Phoomthaisong *et al.*, 2003). After water, among the plant nutrients, nitrogen deficiency is one of the major yield limiting factors for cereals (Shah *et al.*, 2003), hence fertilizer nitrogen application is an essential input for crop productivity in Pakistan. The farmers are facing problem of the capacity of

their soils to supply the quantities of N required (30-80 kg ha<sup>-1</sup>) that is declining rapidly. With continued cereal cropping, fertilizer N must be supplemented with rotations utilizing legumes break crops such as mung bean and mash bean to increase N supply and availability (Strong *et al.*, 1986). As a consequence of the persistent energy crises resulting in higher fertilizer costs, biological N<sub>2</sub>-fixation (BNF) has become one of the most attractive strategies for the development of sustainable agricultural systems. Biological nitrogen fixation occurs mainly through symbiotic association of legumes and some woody species with certain N<sub>2</sub> fixing microorganisms that convert elemental nitrogen into ammonia (Shiferaw *et al.*, 2004). The role of BNF, especially in legumes, is well established and documented. However, it has been reported that various varieties or cultivars of grain legumes show significant differences regarding their ability to support BNF (Hardarson, 1993).

Several of the nutrients that are essential for growth of plants or bacteria play specific roles in symbiotic N<sub>2</sub>-fixation through their effects on nodulation and the N<sub>2</sub>-fixation process (O'Hara, 2001). Phosphorus is one of the most important elements that significantly affect plant growth and metabolism thus its deficiency limits legume production in most agriculture soils (Tang *et al.*, 2001; Abel *et al.*, 2002; Shu-Jie *et al.*, 2007). 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). Mung bean can be successfully grown under limited water supply when at least two irrigations are given 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). Inoculation with suitable rhizobia along with Phosphorus improves symbiotic nitrogen fixation and yield in common bean (Zaman-Allah *et al.*, 2007). Low P availability is especially problematic for leguminous crops, since legume nodules responsible for N<sub>2</sub>-fixation have a high P requirement (Vance, 2001).

Part of the symbiotically fixed N in a legume crop is available to subsequent crops through the decomposition and mineralization of the legume residues. The legume residues can supply more mineral N to the succeeding crops than cereal residues due to their relatively high N contents and relatively low C:N ratio as compared to cereal residues. Research indicates, however, that the N in legume residues is only partially available to plants during the first growing seasons (Wagger, 1989; Stevenson & Vankessel, 1997). Crop residues added to the soil must pass through a microbial biomass that partially mineralize them and partially convert them into new product (van Veen *et al.*, 1984). The residues C and N remaining in the soil are gradually transferred from labile pool to more stabilize pools (Hassink & Dalenberg, 1996). The information regarding the transfer of the residual N into soil organic matter (SOM) fractions, however, is limited. Legume help in solubilizing insoluble P in soil, improving the soil physical environment, increasing soil microbial activity and restoring organic matter and also has smothering effect on weed (Ghosh *et al.*, 2007).

Cereals cropped in sequence with legumes derive N benefits compared with cereal monoculture. N benefits in legume-cereal rotation have been attributed entirely to the transfer of biologically fixed N (Munyinda *et al.*, 1998). An alternative concept is that N benefits are not due to the transfer of fixed N, but can be explained by greater immobilization of nitrate during the decomposition of cereal compared with legume residues (Green & Blackmer, 1995). Others have expressed the view that N benefits may

be due to a combination of legume N-sparing and the transfer of fixed N (Keatinge *et al.*, 1998; Herridge *et al.*, 1995). The decomposition of legume residues during the post harvest fallow period preceding cereal may explain differences in the relative contribution of fixed-N to the N economies of intercropped and rotation systems (Peoples & Herridge, 1990). Agro-economic studies of mung bean-wheat and fallow-wheat cropping systems revealed that water requirement for mung bean growth cycle varied depending upon seasons, potential evapotranspiration and crop coefficients. Wheat growth, development and yield differ significantly when followed after mung bean crop as compared to fallow (Asim *et al.*, 2006).

Mung bean and mash bean have the potential to be included in the present farming system of Pothwar and northern Punjab of Pakistan for improving soil fertility and increasing crop productivity. Hence, there is a need to investigate and evaluate maximum potential of these beans and their residual effect on soil fertility and crop productivity at various phosphorus levels. Keeping in view the importance of grain legume crops and the role they can play in maintaining soil productivity and crop yield, the study was undertaken to quantify N<sub>2</sub>-fixation by mung bean and mash bean using the xylem sap technique and to evaluate their residual effect to the succeeding wheat yield under rainfed conditions.

### Materials and Methods

**Site and experimental description:** Rotational field experiments were conducted on mung bean (*Vigna radiata*) and mash bean (*Vigna mungo*) during summer seasons (2002 and 2003), followed by wheat (*Triticum aestivum*) in each year. The experiments were conducted at Research Farm of University of Arid Agriculture, Rawalpindi (UAAR, 33° 38'N, 73° 04'E) near federal capital Islamabad. Sandy loam soil (Typic Ustochrepts) with an organic C content 0.61 g 100g<sup>-1</sup> and pH of soil paste 7.70 in the top 15 cm depth had developed on moderately coarse textured loess material and is well drained. The site receives an average annual rainfall about 700 mm with summer dominance (70 % between July and September). Meteorological data was collected at Regional Agro Meteorological Center (RAMC, UAAR station) and is presented in Fig 1a & 1b. The experiments were undertaken to assess N<sub>2</sub>-fixation by mung bean and mash bean, WUE of grain and N<sub>2</sub>-fixation and their effect on soil fertility (NO<sub>3</sub>-N) and subsequent wheat yield. The legumes crop i.e. mung bean (Var. NM-92) and mash bean (Var. Mash-3) was sown with seed rate 20 and 18 kg ha<sup>-1</sup>. The legumes were grown with and without Phosphorus fertilizer. Sorghum (Var. YSS-98) was also sown as non-legume crop with seed rate 25 kg ha<sup>-1</sup> and 100kg N ha<sup>-1</sup>. Phosphorus was applied as single super phosphate and nitrogen was applied to sorghum only in the form of urea. Each experiment was replicated four times in a Randomized Complete Block Design. The net plot size was 5×5 m. Treatments were T<sub>1</sub>) Mung bean, T<sub>2</sub>) Mash bean, T<sub>3</sub>) Mung bean + P @ 80 kg ha<sup>-1</sup>, T<sub>4</sub>) Mash bean + P @ 80 kg ha<sup>-1</sup>, T<sub>5</sub>) Sorghum + N @ 100 kg ha<sup>-1</sup>. Legume species were also inoculated at sowing with effective *Bradyrhizobia*. During winter of 2002-03 and 2003-04, the whole fields were under wheat (Var. Wafaq-2001).

**Soil sampling and chemical analysis:** Soil to summer legumes and wheat were sampled two times each year. For NO<sub>3</sub>-N and gravimetric moisture contents, soil samples were taken with king tube from 0-30, 30-60, 60-90, 90-120 cm depths at sowing and

harvesting. NO<sub>3</sub>-N was determined by Salicylic acid method (Vendrell & Zupancic, 1990). The intensity of yellow color was quantified at 410 nm for NO<sub>3</sub>-N determination. Soil NO<sub>3</sub>-N (mg kg<sup>-1</sup>) values were converted to a kg ha<sup>-1</sup> basis using soil bulk density values measured for that growing season. The mean bulk densities for the two experimental years were 1.40, 1.50, 1.60 and 1.65 Mg m<sup>-3</sup> for the 0-03, 30-60, 60-90 and 90-120 cm depth, respectively. Total nitrate-N to a depth of 1.2 m was calculated by summing the values for each of the four sections. Plant available water (mm) was calculated by multiplying gravimetric values with bulk density and core depth.

**Plant sampling:** The legumes plants roots (five) were excavated from 1m<sup>2</sup> 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<sup>2</sup> quadrat in each plot were harvested, oven dried at 80°C to a constant mass, separated into grain and straw then weighed. The remainder of the plot was then cut at ground level and all above ground plant material removed. In Pothwar area of Pakistan, crop residues are commonly removed from the land on which they were produced, to be used as animal feed and fuel. Shoot and grain N concentration were determined by colorimetric procedures (Anderson & Ingram, 1993).

**Assessment of N<sub>2</sub>-fixation by xylem solute technique:** N<sub>2</sub>-fixation was estimated by Xylem Solute Technique. Sap was collected at the pod-fill stage by Vacuum Extraction Method and stored in the freezer at -15°C, then concentrations of ureide, nitrate and amino-N were determined by prescribed methods (Peoples *et al.*, 1989) to calculate the relative abundance of ureide (RUN%) and % P<sub>fix</sub> (proportion of plant N derived from N<sub>2</sub>-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<sub>2</sub>-fixation (%P<sub>fix</sub>) was estimated.

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

**Calculating N<sub>2</sub>-fixed (kg ha<sup>-1</sup>):** The legume N was derived from the measure of biomass accumulation and tissue N-content.

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

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<sub>2</sub>-fixation.

$$\text{Amount of N}_2\text{-fixed (kg ha}^{-1}\text{)} = \%P_{\text{fix}} \times \text{Crop N (kg ha}^{-1}\text{)} \times 1.5^*$$

\*1.5 factor was used to include an estimate for contribution by below ground N (Peoples *et al.*, 1989).

**Water use efficiency of grain yield and N<sub>2</sub>-fixed:** Water use efficiency of grain yield and N<sub>2</sub>-fixation of legumes was calculated by combining grain yield and N<sub>2</sub>-fixation estimates (based on xylem sap) with data on water use (Gregory, 1991 and Herridge *et al.*, 1995).

$$WUE = e / (f - g + h)$$

where e is grain yield (kg ha<sup>-1</sup>) or N<sub>2</sub>-fixed (kg ha<sup>-1</sup>), f and g are soil water contents (mm) to 120 cm depth measured at planting and at harvest, respectively and h is growing season precipitation.

**Statistical analysis:** The data collected for various characteristics were subjected to statistical analysis using Randomized Complete Block Design. Two years data in case of beans and wheat were combined for broad based and 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

**Yield of summer beans:** The data showed that inoculation along with phosphorus fertilizer had a significant effect on nodulation, shoot dry matter, grain yield, %P<sub>fix</sub> and N<sub>2</sub>-fixed in both beans (Table 1). During 1<sup>st</sup> year of experiment, both beans were less affected by applied phosphorus. Shoot dry matter of mung bean showed small increased with phosphorus fertilization; grain yield and grain N were not significantly affected. In the following year of 2003, shoot dry matter, grain and N yield were significantly increased by the application of phosphorus fertilization (Table 1). Highest grain yield (> 1.6 t ha<sup>-1</sup>) and grain N (61 kg ha<sup>-1</sup>) of mash bean were recorded during 2003.

**N<sub>2</sub>-fixation of beans:** During first year, much better nodulation was observed while second year data showed excellent nodulation with phosphorus fertilization and excellent potential for nitrogen fixation. In treatments with phosphorus fertilization, the highest number of nodules occurred which were 24 and 28% higher in mung and mash bean, respectively than in non-fertilized beans. Nodule grade was statistically higher with P fertilization during summer 2003 (Fig. 2). Estimates of nitrogen derived from atmosphere (P<sub>fix</sub>) based on xylem sap solute (ureide, nitrate and amino N), ranged from 40-44 % during first year and from 49-71% during second summer of 2003 (Table 1). At each year %P<sub>fix</sub> was increased by phosphorus fertilization. Both mung and mash beans fixed substantial but variable quantities of nitrogen per hectare. Total N<sub>2</sub> fixed ranged between 13-19 and 54-91 kg ha<sup>-1</sup> during summer 2002 and 2003, respectively (Table 1). N<sub>2</sub> fixation valued showed that mash bean was better N<sub>2</sub>-fixer.

**Water use efficiency of grain yield and N<sub>2</sub>-fixed by beans:** Values of WUE for grain yield during summer 2002 were not affected by phosphorus fertilization (Table 2). However, during second summer WUE based on grain yield were 23-33% higher with phosphorus fertilization. Values of WUE for N<sub>2</sub>-fixation ranged between 0.22 and 1.00 kg ha<sup>-1</sup> mm<sup>-1</sup> and declined with declining %P<sub>fix</sub>. Two years data indicated that values of WUE of N<sub>2</sub>-fixation of mash bean under normal soil fertility conditions was 304% higher than previous year mash bean crop. Similarly, WUE of N<sub>2</sub>-fixation of mash bean fertilized with phosphorus fertilization was 354% higher than previous values (Table 2).

**Table 1. Nodulation, yield and nitrogen fixation of mung bean and mash bean.**

Crops	P level	Nodule	Shoot DM	Grain	Shoot N <sup>A</sup>	Grain N <sup>B</sup>	P <sub>fix</sub>	N <sub>2</sub> fixed <sup>C</sup>
	kg ha <sup>-1</sup>	numbers	t ha <sup>-1</sup>	t ha <sup>-1</sup>	kg ha <sup>-1</sup>	kg ha <sup>-1</sup>	%	kg N ha <sup>-1</sup>
<b>Summer 2002</b>								
Mung bean	0	18	1.70 <sup>N.S</sup>	0.49 <sup>N.S</sup>	30.78 fg	14.25 <sup>N.S</sup>	40.28 <sup>N.S</sup>	18.85 gi
Mash bean	0	20	1.18	1.05	21.24 gh	34.36	41.30	13.33 i
Mung bean	80	20	1.80	0.42	26.25 gh	15.84	42.49	16.58 hi
Mash bean	80	23	1.19	0.77	19.15 h	27.98	43.67	12.9 i
Sorghum		n.d	4.95	n.d	n.d	n.d	n.d	n.d
<b>Summer 2003</b>								
Mung bean	0	38	3.89	0.99	63.42 c	36.02	49.15	54.14 c
Mash bean	0	36	4.27	1.30	84.85 a	46.00	53.82	79.78 b
Mung bean	80	47	4.18	1.32	68.51 bc	48.77	56.69	79.79 b
Mash bean	80	46	4.38	1.66	75.29 ab	60.71	70.89	90.77 a
Sorghum		n.d	11.10	n.d	n.d	n.d	n.d	n.d

<sup>A</sup>(shoot dry matter kg ha<sup>-1</sup>) × (shoot %N)<sup>B</sup>(grain yield kg ha<sup>-1</sup>) × (grain %N)<sup>C</sup>(%P<sub>fix</sub> at pod fill stage) × (shoot N kg ha<sup>-1</sup>) × 1.5Mean 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; <sup>N.S</sup>Non- significantly different (p<0.05) according to Duncan's Multiple Range Test (DMRT)**Table 2. Water use efficiency (kg ha<sup>-1</sup> mm<sup>-1</sup>) of grain and nitrogen fixation of mung bean and mash bean.**

Previous crops	Soil H <sub>2</sub> O (mm)		Total H <sub>2</sub> O Use <sup>A</sup>	WUE (kg ha <sup>-1</sup> mm <sup>-1</sup> )	
	Sowing	Harvest	mm	Grain <sup>B</sup>	N <sub>2</sub> fixation <sup>C</sup>
<b>Summer 2002</b>					
Mung bean	184.50	260.50	613.30	0.84e	0.031 <sup>N.S</sup>
Mash bean	184.50	259.75	614.05	1.72ac	0.022
Mung bean + 80 kg P ha <sup>-1</sup>	184.50	262.27	611.52	0.70e	0.027
Mash bean + 80 kg P ha <sup>-1</sup>	184.50	264.32	609.48	1.25d	0.022
Sorghum + 100 kg N ha <sup>-1</sup>	n.d	n.d	n.d	n.d	n.d
<b>Summer 2003</b>					
Mung bean	239.65	278.39	801.86	1.24d	0.058
Mash bean	239.65	312.54	767.71	1.70ac	0.089
Mung bean + 80 kg P ha <sup>-1</sup>	239.65	279.19	801.06	1.65bc	0.072
Mash bean + 80 kg P ha <sup>-1</sup>	239.65	284.62	795.63	2.09a	0.100
Sorghum + 100 kg N ha <sup>-1</sup>	n.d	n.d	n.d	n.d	n.d

<sup>A</sup>Calculated as sowing soil H<sub>2</sub>O – harvest soil H<sub>2</sub>O + in crop rainfall i.e. summer 2002 689.30mm, summer 2003 840.60mm (see Herridge *et al.*, 1995)<sup>B</sup>Expressed as kg grain ha<sup>-1</sup> mm<sup>-1</sup> H<sub>2</sub>O used; grain yield data in Table 1<sup>C</sup>Expressed as kg N<sub>2</sub>-fixed ha<sup>-1</sup> mm<sup>-1</sup> H<sub>2</sub>O used; N<sub>2</sub>-fixation data in Table 1<sup>N.S</sup>Non- significantly different (p<0.05) according to Duncan's Multiple Range Test (DMRT)

Data is average of 4 replicates; n.d: not determined

**NO<sub>3</sub>-N after summer legumes:** Total NO<sub>3</sub>-N in soil profile (0-120 cm) indicated that higher value 64 and 67 kg ha<sup>-1</sup> were found under mash bean with phosphorus fertilizer at the harvest of summer 2002 and 2003, respectively (Fig. 3). The increase in total NO<sub>3</sub>-N from the previous year was found to be 5%. It was observed that mash bean residual effect was higher and range of total NO<sub>3</sub>-N were between 56-67 kg ha<sup>-1</sup> for legumes and between 40-45 kg ha<sup>-1</sup> for non-legume sorghum. Additional residual soil N under legumes, relative to adjacent sorghum crop i.e., calculated as the difference between soil NO<sub>3</sub>-N under legume and adjacent non-legume sorghum were in the range of 16-22 kg ha<sup>-1</sup>.

**Table 3. Residual effect of mung bean and mash bean on wheat yield.**

Previous crops	Wheat DM t ha <sup>-1</sup>	Wheat grain t ha <sup>-1</sup>
<b>2002-03</b>		
Mung Bean	4.24 <sup>N.S</sup>	2.16 <sup>N.S</sup>
Mash Bean	4.68	2.52
Mung Bean + 80 kg P ha <sup>-1</sup>	4.73	2.58
Mash Bean + 80 kg P ha <sup>-1</sup>	4.40	2.27
Sorghum + 100 kg N ha <sup>-1</sup>	4.20	2.13
<b>2003-04</b>		
Mung Bean	8.58 <sup>N.S</sup>	3.43 <sup>N.S</sup>
Mash Bean	9.15	3.56
Mung Bean + 80 kg P ha <sup>-1</sup>	9.70	3.64
Mash Bean + 80 kg P ha <sup>-1</sup>	9.97	3.76
Sorghum + 100 kg N ha <sup>-1</sup>	7.61	3.07

N.S= Non -significant ( $p < 0.05$ ) according to Duncan's Multiple Range Test (DMRT)

Data is average of 4 replicates

**Yield of following wheat:** Wheat dry matter and grain yield were substantially greater following legumes either fertilized with P or not than following non legume sorghum (Table 3). Maximum wheat DM, during 1<sup>st</sup> year was 4.73 t ha<sup>-1</sup> which tend to increase over 9.90 t ha<sup>-1</sup> during following year of 2003-04. There were also residual benefits for the wheat of fertilizer N applied to the previous sorghum crop. During 1<sup>st</sup> year, the benefits were almost similar in magnitude to the legume benefits; however, during 2<sup>nd</sup> succeeding year, legumes benefits were higher. Grain yield responded similarly with average increase of 20 % during both years.

## Discussion

Shoot dry matter yield of mung bean and mash bean on an average, for both years was 2.99 and 2.78 t ha<sup>-1</sup> with phosphorus fertilization and 2.79 and 2.72 t ha<sup>-1</sup> without phosphorus application, respectively. Phosphorus fertilizer produced 7 and 2% higher biomass yield of mung bean and mash bean, respectively. Phosphorus is important for plant growth and its deficiency limits legume production in most agriculture soils (Tang *et al.*, 2001; Abel *et al.*, 2002; Shu-Jie *et al.*, 2007; Fatima *et al.*, 2007). Non-legume sorghum on average produced 8 t ha<sup>-1</sup> of its dry matter. The data of Table 1 clearly depict that both mung bean and mash bean performed better due to favourable environmental and soil conditions. It may be possible that the well developed root system absorbed more moisture and nutrients from soil as elongated roots cover more surface area in the soil and as such fixed substantial quantity of nitrogen through nodulation on roots. Environmental effects on biomass production e.g., seasonal differences were as or more important than management imposed effect (Keatinge *et al.*, 1988). The biomass yields of both legumes for the following years were significantly higher than the preceding year and at both level of phosphorus application. Overall, 70% improvement was observed which can be interpreted to large extent July-September rainfall (see Fig. 1a & 1b for rainfall data) and integrated residual effect of previous legumes and phosphorus fertilization. However improved management practices during 2<sup>nd</sup> year including proper

seed bed preparation, optimum sowing dates, weeding and application of pesticides etc., increased the dry matter production. This improvement had a great ecological importance. It is expected that if such improvement continues unabated year after year, the over all situation will improve considerably within a few years. The variations in biomass production among the legumes crops can be attributed to differences in genetic potential, plant architecture, rooting pattern and N<sub>2</sub>-fixing capacity as were reported by Mandel *et al.*, (1990). The variation in grain yield production among two legumes can be attributed to differences in genetic potential.

Both beans when fertilized with phosphorus @ 80 kg ha<sup>-1</sup> had the highest number of nodule and nodule grade, which was statistically significant to that in the treatments with 0 phosphorus and the responses were 50-100%. Phosphorus is required for normal functioning in N<sub>2</sub>-fixing bacteria and for effective nodulation on the root system of leguminous crops (Brady & Weil, 2004). Beans %P<sub>fix</sub> values were highest during second succeeding summer and with phosphorus fertilization. These %P<sub>fix</sub> values were similar to those of Ali *et al.*, (1999) who reported that the P<sub>fix</sub> values for soybean, mung bean and mash bean were 16, 31 and 46% in 1996 (1<sup>st</sup> year) and 31, 37 and 48%, respectively during 2<sup>nd</sup> year of 1997. Inoculation with suitable rhizobia with Phosphorus improves symbiotic nitrogen fixation and yield in common bean (Zaman-Allah *et al.*, 2007). Experimental treatments and environmental or nutritional variable have generated a large range of P<sub>fix</sub> values (0-98%) (Peoples *et al.*, 1995). Mash bean, overall had superior dry matter production, highest grain yield and fixed more nitrogen than mung bean during both years, these attributes combined with greater WUE make mash bean a far more suitable legumes to use as a summer crop within a wheat rotation under the prevailing conditions of Pothwar areas. Similar results were also found 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. Data of two years indicated that the WUE of N<sub>2</sub>-fixation of both beans during 2<sup>nd</sup> year was 87- 354% higher than previous year values. These higher values associated with higher %P<sub>fix</sub> (Table 1) during 2<sup>nd</sup> year of summer 2003. Water use and WUE of grain and biomass yields are used to evaluate and compare the efficiency of cropping usually in respect of applied treatments (Gregory, 1991). The concept may have relevance to legume N<sub>2</sub>-fixation (Herridge *et al.*, 1995). Herridge *et al.*, (1995) calculated WUE of chickpea N<sub>2</sub>-fixation to vary between 0.14 and 0.24 kg ha<sup>-1</sup> mm<sup>-1</sup>, with the higher values associated with higher %P<sub>fix</sub>. Herridge *et al.*, (1995) recommended that additional studies are needed to provide a greater range of %P<sub>fix</sub> values, particularly at high values, to determine an upper limit of efficiency may be about 0.40 kg N<sub>2</sub>-fixed ha<sup>-1</sup>mm<sup>-1</sup>. Once the upper limit of efficiency is determined, evaluation of legumes N<sub>2</sub>-fixation in term of WUE may prove valuable.

Maximum values of NO<sub>3</sub>-N of soil profile (0-120 cm) were observed under mash bean fertilized with phosphorus during both years and lowest values were under non-legume sorghum. Phosphorus along with rhizobium inoculation increased nitrogenase activity as well as improved soil fertility for sustainable agriculture (Gentili & Huss-Danell, 2003; Fatima *et al.*, 2007). Nodulation and N<sub>2</sub>-fixation may directly affect nitrate uptake and metabolism (Wyeh & Rains, 1978). Nitrate is also isolated in the soil through restricted root growth, particularly deep in profile (Marcellos *et al.*, 1998). Nitrate sparing is a consistent trait of legumes in rotation system and this nitrate sparing may be due to nodulation induced retardation of root growth of the legume, which induces sites for absorption of mineral N (Marcellos *et al.*, 1998).

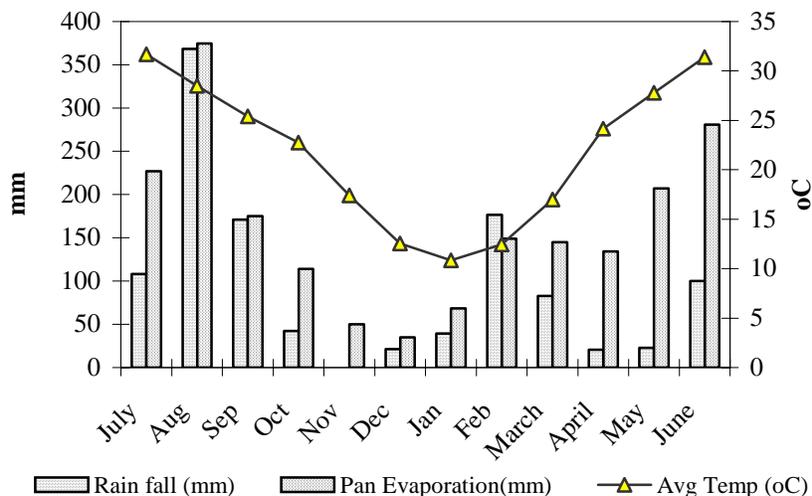


Fig. 1a. Rainfall (mm), pan evaporation (mm) and temperature (°C) during 2002-03.

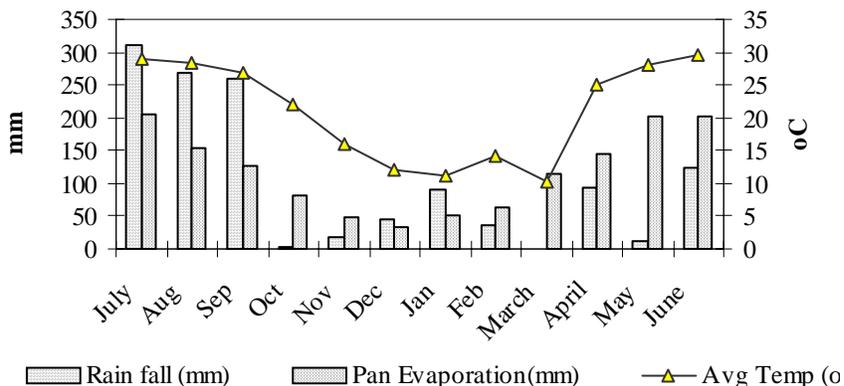


Fig. 1b. Rainfall (mm), pan evaporation (mm) and temperature (°C) during 2003-04.

Beans with phosphorus fertilization increased grain yield of succeeding wheat by 20% over non-legume sorghum. The wheat grains were also 11% higher in plots previously under mung baen, fertilized with phosphorus as compared to beans without phosphorus. The wheat grain yield on average 2.60 t ha<sup>-1</sup> was lowest in sorghum-wheat sequence. Yield of cereal grown after legumes is generally increased often as much as by 80% compared with cereal grown after cereal. (Badr-ud-din & Mayer, 1989). Improvement in cereal yield of following monocropped legumes are in the range of 0.5 to 3 t ha<sup>-1</sup> representing around 30 to 35% increase over yield obtained *via* cereal cropping sequences (Peoples & Crasswell, 1992; Wani *et al.*, 1995). Mung bean fits very well in the rainfed agro ecosystem as it helps water conservation and nutrient recycling for the total system. Wheat growth, development and yield differ significantly when followed after mung bean crop as compared to fallow (Asim *et al.*, 2006).

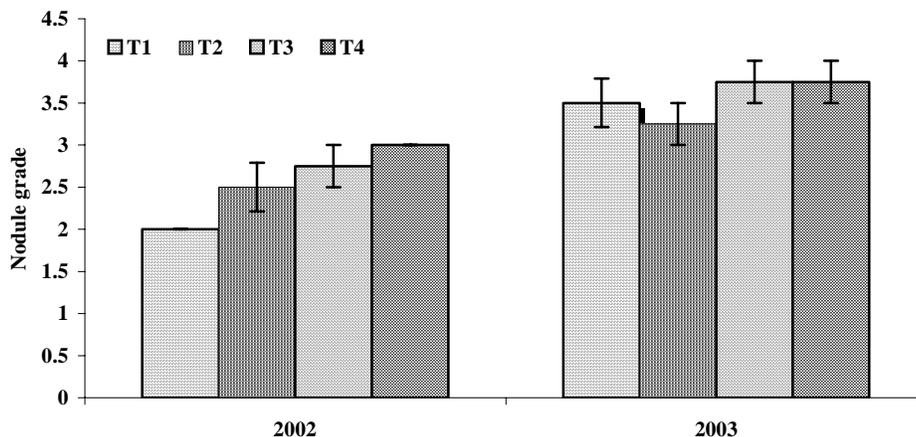


Fig. 2. Nodule grading of mung bean and mash bean.  
 T<sub>1</sub> Mung bean, T<sub>2</sub> Mash bean, T<sub>3</sub> Mung bean + P @80 kg ha<sup>-1</sup>, T<sub>4</sub> Mash bean+ P @80 kg ha<sup>-1</sup>

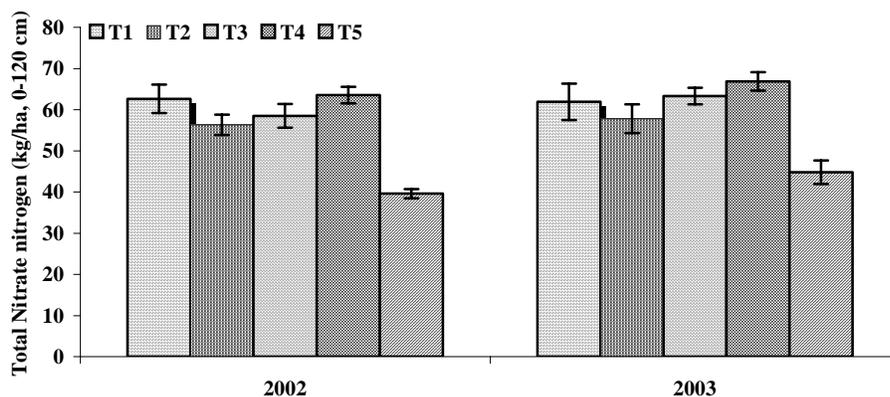


Fig. 3. Total NO<sub>3</sub>-N after harvest of summer crops to a depth of 1.2 m.  
 T<sub>1</sub> Mung bean, T<sub>2</sub> Mash bean, T<sub>3</sub> Mung bean + P @80 kg ha<sup>-1</sup>, T<sub>4</sub> Mash bean+ P @80 kg ha<sup>-1</sup>

### Conclusion

N<sub>2</sub>-fixation capacity of mung and mash beans was enhanced by application of phosphorus fertilization. Legume-cereal sequence also enhanced biomass and grain yield of subsequent wheat. However, it is recommended that further long term trials involving legumes-cereal rotation may be conducted on farmer's field to get comprehensive data, improving the understanding and to come up to a more sound conclusion.

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