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NITROGEN FIXATION OF LEGUMES AND YIELD OF WHEAT UNDER LEGUMES-WHEAT ROTATION IN POTHWAR

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Abstract

Legumes build soil fertility and contribute substantial amounts of N for sustainability of cereal based cropping systems. These lacking information for the system as a whole in rainfed pothwar were investigated through field experiments at two different locations i.e. (Research farm of University of Arid Agriculture Rawalpindi, UAAR and farmer's fields in Chakwal district). Mung bean (Var. NM-92) and mash bean (Var. Mash-3) were rotated with wheat (Var. Wafaq 2001) with the objectives to assess N_2-fixation of these legumes and study their residual effects on soil NO_3-N and subsequent wheat yield. Sorghum (Var. YSS-98) was also grown as non-legume reference crop. Mash bean not fertilized with P produced 4.27 t ha^{-1} of dry matter compared with 4.38 t ha^{-1} when fertilized with P @ 80 kg ha^{-1}. Similarly, mash bean fertilized with P yielded 4% more grain followed by mung bean and response of P fertilizer was 13%. Legumes N_2-fixed ranged from 13-80 and 18-38 kg ha^{-1} at UAAR and Chakwal site, respectively. The NO_3-N contents in the soil under non-legume sorghum were less compared with legumes. Maximum contents of total NO_3-N, 58 kg ha^{-1} in soil profile was observed under mash bean fertilized with P. Additional residual soil NO_3-N under legumes relative to adjacent sorghum crop were 22 kg ha^{-1} at UAAR site, 83 % higher when compared to Chakwal site. Both legumes with and with out P fertilization increased the biomass and grain yield of succeeding wheat with an increase of 18% over non-legume sorghum. Legumes-cereal sequence improved NO_3-N status of soil as well as yield of subsequent wheat.

Introduction

Pothwar plateau of the northern Punjab are largely deficient in nutrients especially B (Rashid *et al*., 1997). The economic using of P, B and Zn in legumes was proven very attractive from growers point of view (Din *et al*., 2001).The farmers marginally grow legumes without fertilizer application and without realizing the carry-over effect of the legume on the following wheat. In some situations, the fertilizer application in legumes results in taller plants and excessive vegetative growth, leading to poor pod development and reduced yield. Imbalanced use of NPK fertilizers and virtually very little use of organics by the farmers in Pothwar result in poor yields of wheat as well as deterioration of soil fertility. Usually the farmers in Pothwar area leave their land fallow during summer and only ploughed the fields to conserve soil moisture for wheat production during winter seasons.

Contribution of legumes towards N economy in cereal-based cropping systems is well-known (Sharma & Behera, 2009). In crop rotations, grain legumes contribute to a diversification of cropping systems and as N_2-fixing plant it can reduce the mineral N fertilizer demand (Mayer *et al*., 2003). Generally in sustainable and organic farming systems, biological N_2-fixation by legumes is the main source of nitrogen for the crop rotation. Hence cropping of grain legumes is a prioritized area of research in the rainfed areas. Grain legumes cause significant, positive yield effects on subsequent non-legumes when compared with rotations with non-legumes (Chalk, 1998; Sanginga, 2003). In addition to its beneficial factors, such as improving soil structure, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, nitrogen is a key factor in the positive response of cereals following legumes (Chalk, 1998). However the improvement in N nutrition of nonfixing crops in grain legume-based cropping systems requires a more fundamental understanding of the decomposition of grain legume residue processes and the interactions with soil organic matter. Grain legume species and varieties growing at the same location differ significantly in dry matter production, N accumulation, N_2-fixation, N-balance and residue quality (Beck *et al*., 1991; Haynes *et al*., 1993; Evans *et al*., 2001). These differences may be the main factors determining the residual N contribution to subsequent crops (Hood *et al*., 1999).

In this paper and the previous one (Hayat *et al*., 2008), we examined rotational effects of mung and mash bean at two sites in Pothwar, northern Punjab, Pakistan in which legumes (mung and mash beans) in summer season were followed by wheat in subsequent season of the same year. Inclusion of legumes increases soil fertility and consequently the productivity of succeeding cereal crops (Ghosh *et al*., 2007). Kumar & Prasad (1999) reported a saving of 25 kg N ha^{-1} in wheat when grown after a grain legume. The nitrogen economy was affected not only due to direct N addition through legume residues and its subsequent mineralization but also due to enrichment of soil with fixed N_2 from root exudates (Pawar & Jadhav, 1995). Such information is lacking for the system as a whole on the rainfed Pothwar, northern Punjab. The present study reports the N_2-fixation of mung and mash beans at two different locations of Pothwar and their residual effects on soil NO_3-N and yields of succeeding rainfed wheat.

Materials and Methods

Field experiments were conducted on mungbean (Var. NM-92) and mashbean (Var. Mash-3) during summer seasons (2002 and 2003), followed by wheat (Var. Wafaq 2001) in each year at two different locations of Pothwar area (Research Farm of UAAR and farmer's field in Chakwal district) to assess N_2-fixation by mung bean and mash bean and study their effect on soil NO_3-N and subsequent wheat yield. The legumes were grown with and without P-fertilizer. Sorghum (Var.YSS-98) was sown as non-legume crop. The legume crops i.e., mung bean and mash bean were sown with seed rate 20 and 18 kg ha^{-1}. Sorghum (Var.YSS-98) was also sown as non-legume crop with seed rate 25 kg ha^{-1} and 100kg N ha^{-1}. 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 5m×5m. Treatments were (1) T_1 Mungbean (2) T_2 Mashbean (3) T_3 Mungbean + P @ 80 kg ha^{-1} (4) T_4 Mashbean + P @ 80 kg ha^{-1} (5) T_5 Sorghum + N @ 100 kg ha^{-1}. Legumes species were also inoculated at sowing with effective *Rhizobia* spp. During winter of 2002-03 and 2003-04, the whole fields were under wheat (Var. Wafaq-2001).

Before commencement of the experiment in 2002-03, soil samples were collected from 0-15 cm profile depth at four places in the experimental field using a core sampler. The soils of the experimental sites were sandy loam (15% clay, 14% silt, 71% sand at UAAR & 16% clay, 6% silt, 78% sand at Chakwal site). Samples were thoroughly mixed and a representative sample was drawn for chemical analysis. The soil of the UAAR field at the start of experiment was non-saline (EC 0.25 d Sm^{-1}), pH 7.7 and contained 0.61 $g100g^{-1}$ total organic carbon, 6.5 ug g^{-1} available P and 3.84 ug g^{-1} NO_3-N. Similarly, the soil of the Chakwal district was also non-saline (EC 0.17 d Sm^{-1}), mildly towards alkaline pH 8.0 and contained 0.34 $g100g^{-1}$ total organic carbon, 3.40 ug g^{-1} available P and 3.02 ug g^{-1} NO_3-N. For NO_3-N, soil samples were taken with king tube from 0-30, 30-60, 60-90, 90-120 cm depths after summer (legumes) harvesting. NO_3-N was determined by salicylic acid method (Vendrell and Zupancic, 1990). The intensity of yellow color was quantified at 410 nm for NO_3-N determination. Soil NO_3-N (mg kg^{-1}) values were converted to a kg ha^{-1} basis using soil bulk density values measured for that growing season. The mean bulk densities for the two experimental years at UAAR and Chakwal fields were 1.40, 1.50, 1.60, 1.65 and 1.55. 1.60, 1.68 & 1.70 Mg m^{-3} for the 0-03, 30-60, 60-90 and 90-120 cm depth, respectively. Total NO_3-N to a depth of 1.2 m was calculated by summing the values for each of the four sections.

The legume plants 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°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 fields on which they were produced, to be used as animal feed and fuel (Hayat *et al*., 2008). Shoot and grain N concentration were determined by colorimetric procedures (Anderson & Ingram, 1993). N_2-fixation was estimated by Xylem Solute Technique (Peoples *et al.*, 1989). The data collected for various characteristics were subjected to statistical analysis using Randomized Complete Block Design. Two years data in case of legumes and wheat were combined for broad based and reliable results. A software package MStat C was used to calculate ANOVA tables and treatments means were separated by using DMRT at $p<0.05$.

Results and Discussion

Yield and N_2-fixed by legumes: Both legumes produced highest number of nodules with P fertilization, which was 33-50% higher than without P (Table 1). Highest biomass of mash bean, 4.38 t ha^{-1} was obtained with 80 kg P ha^{-1} at UAAR site during 2nd year of experiments. Mash bean yielded 31% more grain when compared to mung bean and the response of P fertilization was 28%. Highest shoot N yield, 84.85 kg N ha^{-1}, was observed in mash bean (Table 2). Proportion of nitrogen derived through N_2-fixation ($\%P_{fix}$) in mash bean was 71, 25% higher when compared with mung bean, 57 under P fertilization at UAAR site during 2nd year of experiments (Table 3). N_2-fixation by mash bean with P was 38% higher as compared to mung bean fertilized with P. N_2-fixed by legumes at UAAR site, was 41% higher as compared to Chakwal site and in summer 2003, N_2 fixation was 161% higher than that of previous summer of 2002. It is an admitted fact that nodule formation requires proper inoculation of rhizobia and for normal functioning of rhizobia, phosphorus is required. That is why inoculation along with phosphorus produced maximum number of nodules. Nodulation response of mung bean and mash bean as reflected by the number of nodules per plant exhibited significant improvement as a result of the application of phosphorus and inoculation. It is clear from the overall results that treatment receiving phosphorus fertilizer produced high nodule grade and the responses were upto 50%. Phosphorus is required for normal functioning in N_2-fixing bacteria and for effective nodulation on the root system of leguminous crops (Brady & Weil, 2004). The variation in grain yield production among two legumes can be attributed to differences in genetic potential. Several environmental factors including drought, temperature and soil nutrient status dramatically affect the process at molecular/functional level and thus play a part in determining the actual amount of nitrogen fixed by a given legume in the field (Serraj, 2004).

Table 1. Nodulation and yield of legumes.

Treatments	Locations	Nodules	Dm	Grain	Nodules	Dm	Grain
			t ha^{-1}	t ha^{-1}		t ha^{-1}	t ha^{-1}
		2002			2003		
Mung bean	UAAR	18	1.70	0.49	38	3.89	0.99
Mash bean		20	1.18	1.05	36	4.27	1.30
Mung bean + 80 kg P ha^{-1}		20	1.80	0.42	47	4.18	1.32
Mash bean + 80 kg P ha^{-1}		23	1.19	0.77	46	4.38	1.66
Sorghum + 100 kg N ha^{-1}		n.d	n.d	n.d	n.d	n.d	n.d
$LSD_{0.05}$		3.735	1.369	0.2861	N.S	3.258	0.4047
Mung bean	Chakwal	17	2.76	0.72	20	3.04	0.81
Mash bean		24	2.84	0.80	26	3.08	0.86
Mung bean + 80 kg P ha^{-1}		33	2.87	0.81	32	3.24	0.87
Mash bean + 80 kg P ha^{-1}		34	3.02	0.85	38	3.20	0.88
Sorghum + 100 kg N ha^{-1}		n.d	n.d	n.d	n.d	n.d	n.d
$LSD_{0.05}$		8.435	0.9841	0.08761	N.S	0.9937	N.S

Table 2. Shoot and grain N concentration of legumes.

Treatments	Locations	Shoot N	Grain N	Shoot N yield[a]	Shoot N	Grain N	Shoot N yield[a]
		%	%	kg ha^{-1}	%	%	kg ha^{-1}
		2002			2003		
Mung bean	UAAR	1.81	2.91	30.78	1.64	3.64	63.78
Mash bean		1.80	3.27	21.24	1.99	3.54	84.85
Mung bean + 80 kg P ha^{-1}		1.46	3.77	26.25	1.64	2.94	68.51
Mash bean + 80 kg P ha^{-1}		1.61	3.63	19.15	1.72	3.66	75.29
Sorghum + 100 kg N ha^{-1}		n.d	n.d	n.d	n.d	n.d	n.d
$LSD_{0.05}$				N.S			12.35
Mung bean	Chakwal	1.37	3.29	37.80	1.52	3.03	46.14
Mash bean		1.51	3.28	42.82	1.59	3.14	48.99
Mung bean + 80 kg P ha^{-1}		1.39	3.34	39.91	1.52	3.20	49.20
Mash bean + 80 kg P ha^{-1}		1.54	3.29	46.51	1.64	3.26	52.37
Sorghum + 100 kg N ha^{-1}		n.d	n.d	n.d	n.d	n.d	n.d
$LSD_{0.05}$							

[a](shoot dry matter kg ha^{-1}) × (shoot %N)

Table 3. N_2-fixation and grain off take by legumes.

Treatments	Locations	P_{fix}	N_2-fixed[b]	Grain off take[c]	P_{fix}	N_2-fixed[b]	Grain off take[c]
		%	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1}	kg ha^{-1}
		2002			2003		
Mung bean	UAAR	40.28	18.59	14.25	49.15	47.02	36.02
Mash bean		41.30	13.16	34.36	53.82	68.49	46.00
Mung bean + 80 kg P ha^{-1}		42.49	16.73	15.84	56.69	58.26	48.77
Mash bean + 80 kg P ha^{-1}		43.67	12.54	27.98	70.89	80.05	60.71
Sorghum + 100 kg N ha^{-1}							
$LSD_{0.05}$		N.S	N.S		N.S		
Mung bean	Chakwal	32.23	18.27	23.70	42.99	29.75	24.54
Mash bean		35.23	22.63	26.21	43.02	31.61	27.00
Mung bean + 80 kg P ha^{-1}		32.74	19.60	27.09	46.33	34.19	27.86
Mash bean + 80 kg P ha^{-1}		37.57	26.21	27.92	48.66	38.22	28.67
Sorghum + 100 kg N ha^{-1}							
$LSD_{0.05}$		N.S			N.S	N.S	

[b](%P*fix* at pod fill stage) × (shoot N kg ha^{-1}) × 1.5

[c](grain yield kg ha^{-1}) × (grain %N)

Effect of legumes on soil NO_3-N: The data on soil NO_3-N (Fig. 2a) at UAAR site, after the harvest of summer 2002 shows that maximum NO_3-N (23 kg ha^{-1}) were under mash bean with P fertilization and the increase to non-legume sorghum was 48% at 30-60 cm depth. Similarly, after harvest of summer 2003 (Fig. 2b) mash bean fertilized with P yielded 36% more NO_3-N per hectare when compared with non-legume sorghum at the same depth. The NO_3-N contents in the soil under non-legume sorghum were less compared with legumes. Sorghum depleted the soil NO_3-N and at all depths its concentration was lower as compared with mung and mash beans. The results are in line with those of Blumenthal *et al*., (1988) who reported that various legume crops maintained/improved nitrogen contents of soil through nitrogen fixation. Data on soil NO_3-N at Chakwal site, in 2002 (Fig. 2c) also shows similar trend. The concentration of soil NO_3-N in 2003, at 30-60 cm depth (Fig. 2d) was 42% higher with mash bean under P fertilization than non-legume sorghum. However, at 60-90 cm depth mung bean fertilized with P gave 24% higher value as compared to non-legume sorghum. The soil NO_3-N concentration under mung bean was higher as compared with mash bean at Chakwal site. The probable reason is the higher plant population of mash bean, higher biomass production and more N_2-fixed by mash bean with greater accumulation of NO_3-N for the soil. Management practices that decreased the relative use of soil N by legumes were narrow spacing and/or high plant population density. The theory substantiating these observations is that high plant densities will more rapidly deplete soil nitrate and favour earlier establishment of a functioning symbiosis (Bushby & Lawn, 1992). It was concluded that maximum NO_3-N retained at 30-60 cm depth followed by 60-90 cm soil profile depth except Chakwal site where most of the nitrate remained below 60 cm during summer 2002. By examining soil NO_3-N status by depth, Miller *et al*., (2003) showed that crop differed primarily below the 61 cm soil depth. This indicates that post harvest differences among crops in soil NO_3-N status might be due to differences in root length. This observation is some what consistent with the soil NO_3-N use results (Fig. 2a,b,c,d). When taken as profile (0-120 cm) NO_3-N, maximum values of total NO_3-N (63-67 kg NO_3-N ha^{-1}) were observed under mash bean fertilized with P during both years at UAAR site and lowest values were under non-legume sorghum (Fig 3). Total NO_3-N in soil profile at Chakwal site indicated that on average higher value 57 kg ha^{-1} were found under mash bean with and without P fertilizer, however, at the harvest of summer 2003, mung bean residual effect was higher and range of total NO_3-N at Chakwal site were between 50-58 kg ha^{-1} for legumes and between 40 and 46 kg ha^{-1} for non-legume sorghum. Additional residual soil N under legumes, relative to adjacent sorghum crop i.e. calculated as the difference between soil NO_3-N under legume and adjacent non-legume sorghum were in the range of 16-22 and 10-12 kg ha^{-1} at UAAR site and Chakwal site respectively. Nitrate sparing appears to be a consistent trait of legumes in rotation system (Marcellos *et al*., 1998). Nitrate sparing may be due to nodulation induced retardation of root growth of the legume, which induces sites for absorption of mineral N (Rigaud, 1981). Alternately nodulation and N_2 fixation may directly affect nitrate uptake and metabolism (Wyeh & Rains, 1978). A third possibility is that nitrate is isolated in the soil through restricted root growth, particularly deep in the profile, making comparisons that include those depths invalid (Marcellos *et al*., 1998).

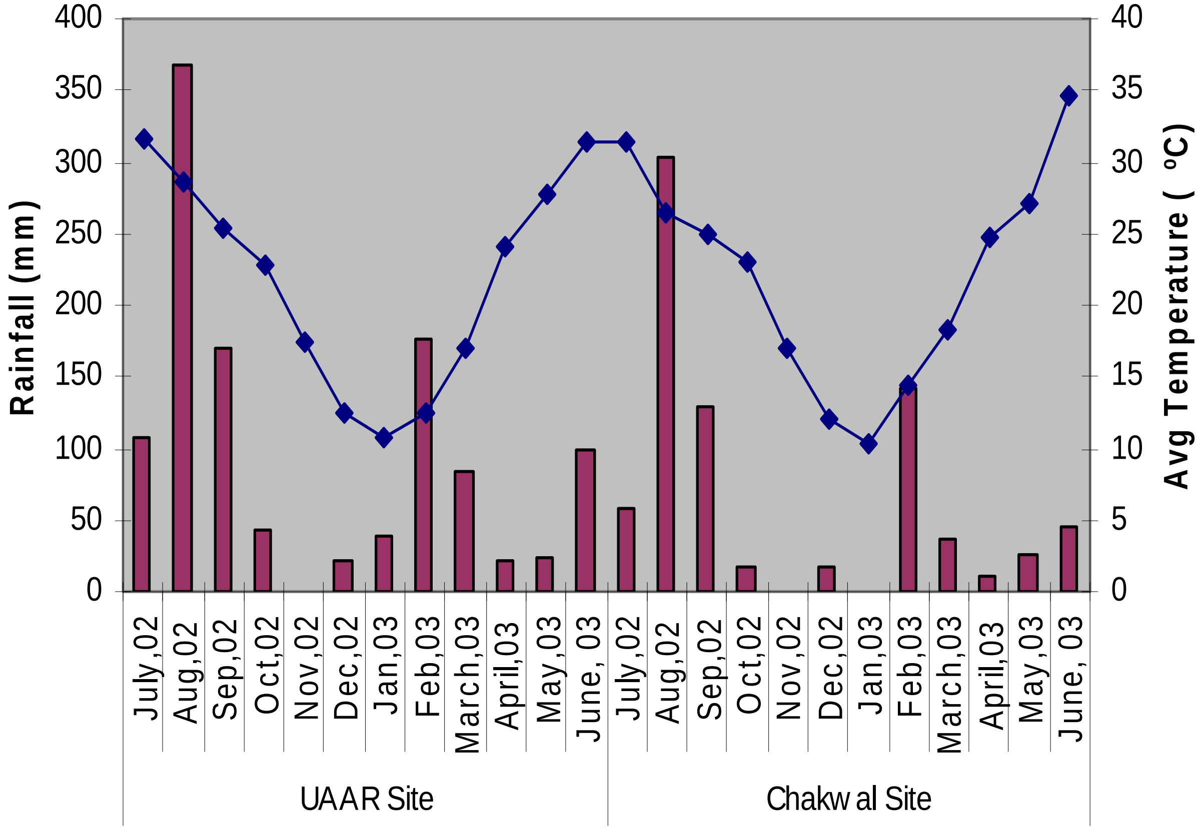

Fig. 1a. Rainfall (mm) and temperature (°C) regime during the year 2002-03 at two sites.

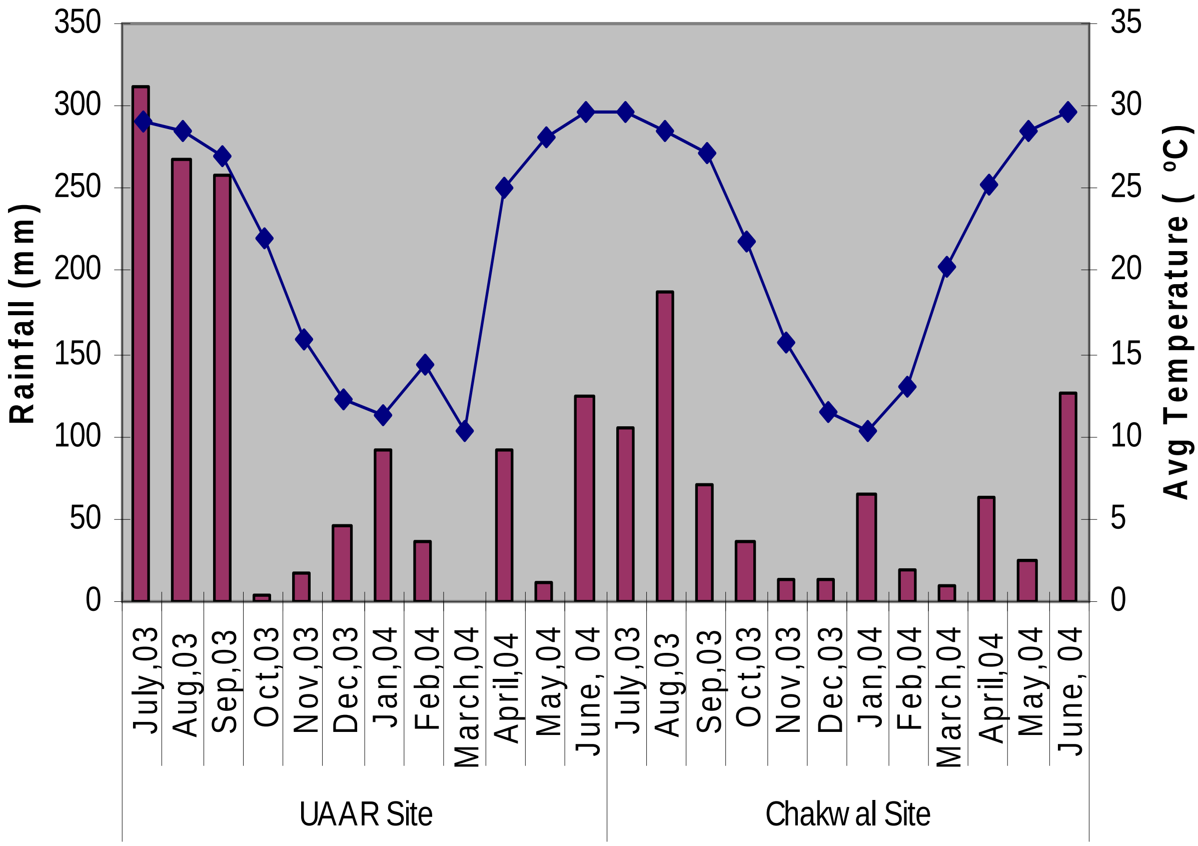

Fig. 1b. Rainfall (mm) and temperature (°C) regime during the year 2003-04 at two sites.

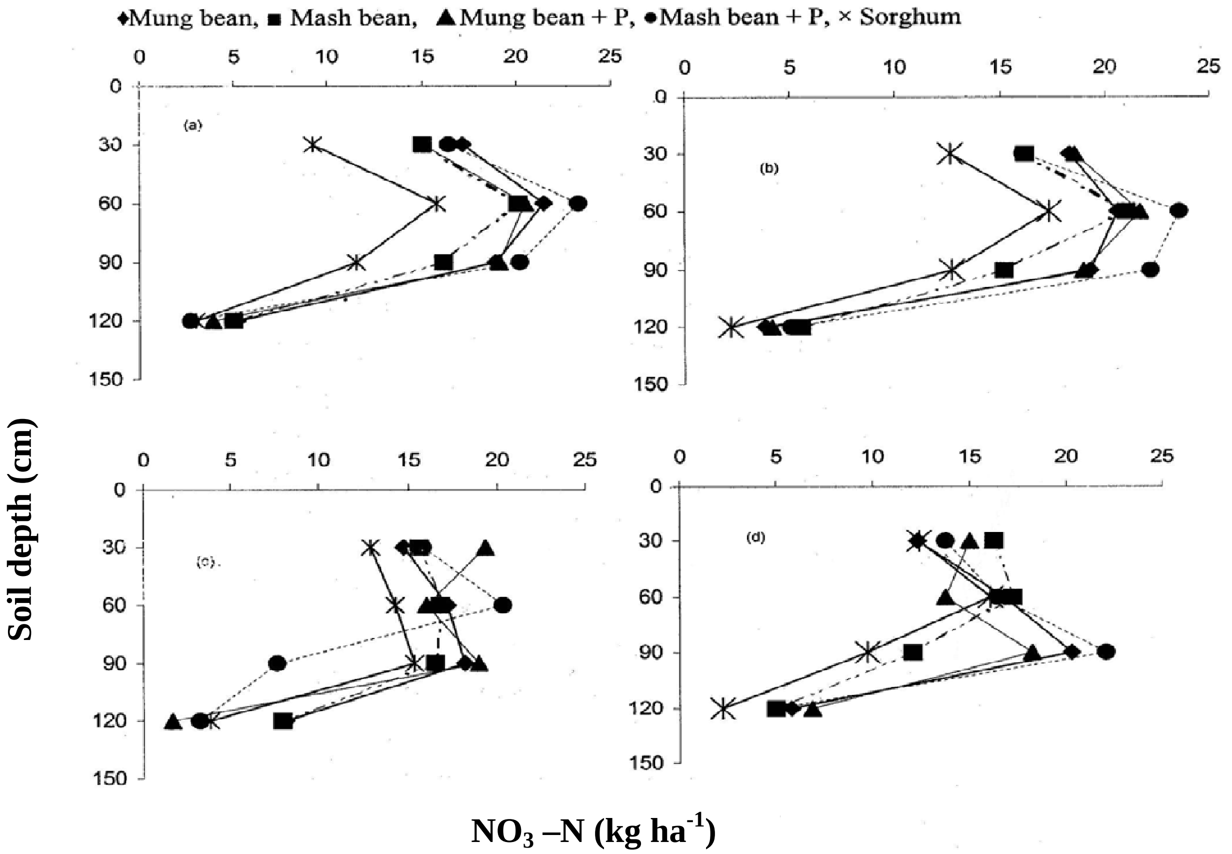

Fig. 2. Distribution of soil NO_3-N with depth. (a) UAAR-2002, (b) UAAR-2003, (c) Chakwal 2002, (d) Chakwal-2003.Each point is the mean of 4 replicates. Data are after harvesting of summer crops.

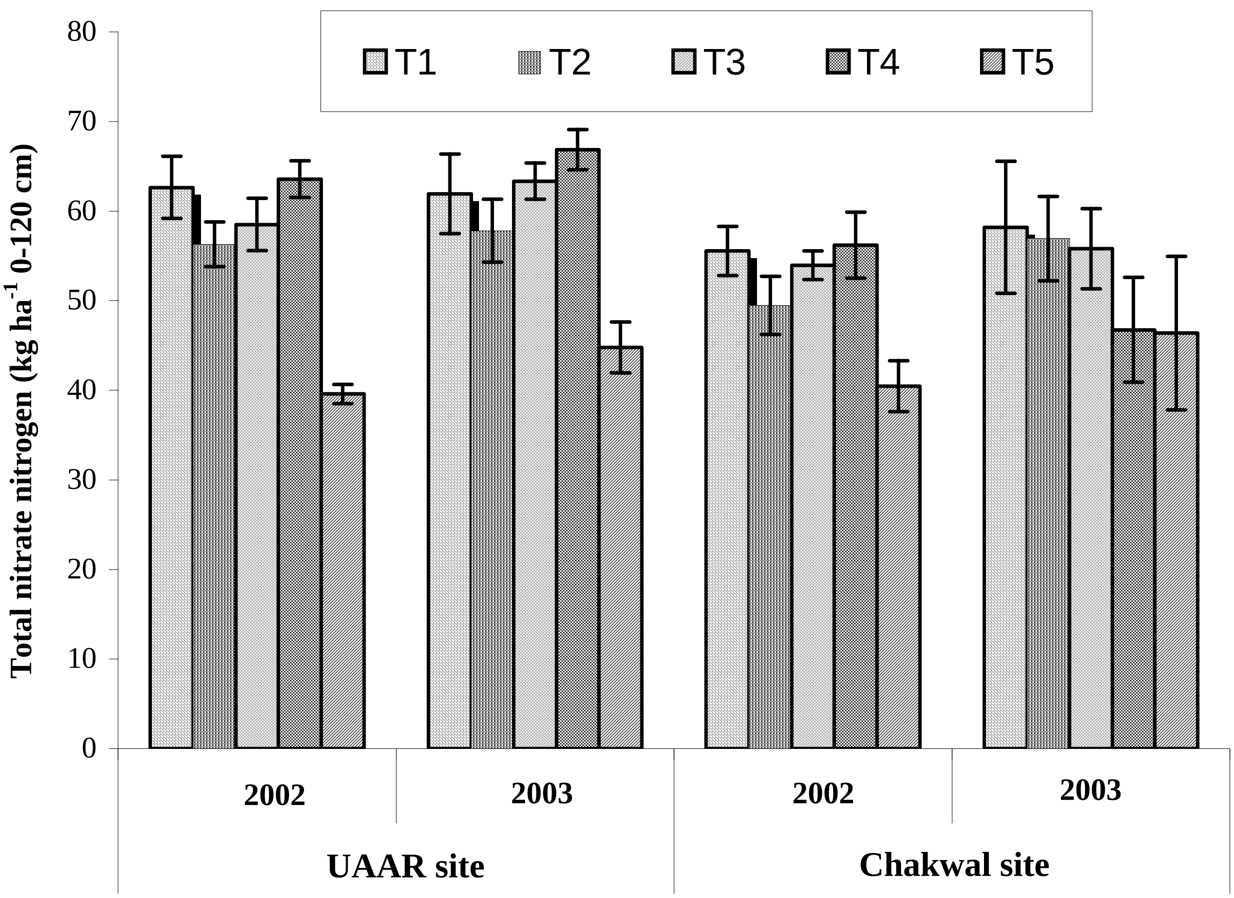

Fig. 3. Total NO_3-N (kg ha^{-1}) to a depth of 1.2 m.

Table 4. Residual effects of legumes and non-legume sorghum on succeeding wheat yield.

Treatments/previous crops	Locations	DM $t\ ha^{-1}$	Grain $t\ ha^{-1}$	DM $t\ ha^{-1}$	Grain $t\ ha^{-1}$
		2002-03		2003-04	
Mung bean	**UAAR**	$4.24^{N.S}$	2.16	8.58 ab	3.43
Mash bean		4.68	2.52	9.15 ab	3.56
Mung bean + 80 kg P ha^{-1}		4.73	2.58	9.70 a	3.64
Mash bean + 80 kg P ha^{-1}		4.40	2.27	9.97 a	3.76
Sorghum + 100 kg N ha^{-1}		4.20	2.13	7.61 b	3.07
$LSD_{0.05}$		0.6786	N.S	1.549	N.S
Mung bean	**Chakwal**	2.58	1.55	5.34	2.92
Mash bean		3.19	1.73	5.37	3.25
Mung bean + 80 kg P ha^{-1}		3.29	1.78	5.40	3.13
Mash bean + 80 kg P ha^{-1}		3.84	1.66	5.34	4.06
Sorghum + 100 kg N ha^{-1}		3.10	1.28	5.07	2.87
$LSD_{0.05}$		N.S	N.S	N.S	N.S

Mean followed by the same letter (s) are not significantly different ($p<0.05$) according to Duncan's Multiple Range Test (DMRT); $^{N.S}$Non- significantly different ($p<0.05$); Data is average of 4

Residual effect of legumes on succeeding wheat yield: The maximum dry matter, 4.73 t ha^{-1}, was observed in plots which were previously under mung bean fertilized with P closely followed by mash bean without P, 4.68 t ha^{-1} at UAAR site, during 1st year (Table 4). Mung bean with phosphorus fertilization increased wheat dry matter by 13 % over non-legume sorghum. The following year at UAAR site, maximum wheat dry matter, 9.97 t ha^{-1}, was observed in the plots which were previously under mash beans fertilized with phosphorus and increase over non-legume sorghum was 31%. The wheat dry matter was lowest in sorghum-wheat sequence. Similar results were observed at Chakwal site. These results are in agreement to those reported by Reeves *et al*., (1984); Evans *et al*., (1991); Oikeh *et al*., (1998); Dalal *et al*., (1998) that annual crop legumes, grown in rotation with cereal crops, can improve yields of the cereals and reported yield responses to previous legume crops are mainly in range of 50-80% increases over yields in cereal-cereal sequences. By comparing both locations, the data on wheat biomass yield reveal that wheat biomass at UAAR site was 58 % higher when compared with Chakwal site. This higher yield is associated with positive residual effect of legumes along with environmental and location differences.

The maximum grain yield, 4.06 t ha^{-1}, was observed in plots which were previously under mash bean fertilized with P closely followed by mung bean without P, 3.25 t ha^{-1} (Table 4) at Chakwal site, during 2nd year of experiments. Mash bean with phosphorus fertilization increased grain yield of wheat by 41 % over sorghum. The yield of cereals grown after legumes is generally increased often as much as by 80% compared with cereals grown after cereals. Legumes in cropping system maintain the soil fertility and leave more moisture in sub-soil as it requires much less amount of water, nutrients and are short-duration compared to cereals (Srivastva *et al.*, 1985; Badr-ud-din & Mayer, 1994). The data on subsequent wheat grain yield (Table 4) clearly depict that during 2nd proceeding winter of 2003-04, grain yield was 71 % higher than previous winter of 2002-03. These results are in accordance with the data of N_2 fixed by preceding legumes crops and biomass of the same wheat crop at both years. The present data reveals that maximum grain yields in this farming system was possible by combining legumes and management practices including application of phosphorus and use of inoculums to the preceding legumes crops. Higher grain yields of cereals after legumes than after a cereal crop were explained largely in terms of additional nitrate-N following the legume crop (Felton *et al.*, 1998; Ding *et al.,* 1998). Improvement in cereal yield following monocropped legumes are in the range of 0.5 to 3 t ha^{-1}, representing around 30 to 35% increase over yield obtained *via* cereal cropping sequences (Peoples & Crasswell, 1992; Wani *et al.*, 1995.

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