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Potential of Summer Legumes to Fix Nitrogen and Benefit Wheat Crop under Rainfed Condition

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Abstract: Rotational field experiments were conducted at University of Arid Agriculture, Rawalpindi Research Farm to investigate the residual effect of summer legume species in wheat-based cropping system in rainfed environment. Legumes species including Soybean (cultivar-NARC 1), Mungbean (NM 92), Mashbean (NARC 2) grown along with maize (Neelam) as reference crop were planted for N₂-fixation determination during summer season of 1998 (July-October) with the aim of evaluating their biomass production and grain yields, nitrogen (N) and N₂-fixation using xylem solute and natural ¹⁵N abundance techniques and the quantification of grain yield differences of succeeding wheat crop compared with a cereal-cereal rotation. During winter wheat crop, Var. Inqalab 91 was grown with 0 and 100 kg N ha⁻¹ on the same plots previously having legumes. Cultivation of summer legume crops therefore, constitutes an alternative to traditional cropping practices of leaving the land fallow. Performance varied greatly between species. Legumes dry matter ranged from 7.46, 3.39 and 4.52 t ha⁻¹ with soybean, mungbean and mashbean, respectively. Maize not fertilized with N produced 9.76 t ha⁻¹ of dry matter compared with 13.56 t ha⁻¹ when fertilized. Mean grain yields for three legumes were 1.68 (soybean), 0.82 (mungbean) and 1.83 t ha⁻¹ (black gram). Maize not fertilized with N produced 4.35 t ha⁻¹ of grains compared with 5.86 t ha⁻¹ when fertilized. The comparison of %Pfix (proportion of plant N derived from N₂-fixation) both (%Run) and (δ¹⁵N) showed that the xylem solute and natural ¹⁵N abundance techniques have independent estimates of %Pfix. Consequently crop Nitrogen fixed ranged from 55-207 and 41-145 kg ha⁻¹ by xylem solute and δ¹⁵N techniques, respectively. Data were also subjected to simple regression analysis with Pfix and total N₂-fixed as the dependent variables and shoot N, dry matter and grain yield as independent variables both for % RUN and δ¹⁵N. Regression coefficient (r²) for Pfix of xylem solute were 0.45 (p<0.05) with shoot N as the independent variable and 0.63 with grain yield. For total N₂-fixed, the regression coefficient was high at 0.87 using dry matter yield. When N₂ fixation was calculated by δ¹⁵N data, regression coefficient (r²) for Pfix were 0.21 and 0.59 with shoot N and grain yield, respectively. However, for total N₂-fixed comparatively high value of regression coefficient (r² = 0.80; p<0.05) was obtained with biomass yield. Both legume rotation and fertilizer N improved the biomass and grain yield of following wheat crop. For 0N wheat increase were 2.5 and 3.6% for wheat biomass, when the prior crops were soybean and mungbean and 7 and 14% for grain yield, respectively. The response of the wheat to fertilizer N, irrespective of prior crop, were large, ranging from 5 to 11%. The results highlight the need for thorough biological economic analysis of cropping option.

Key words: Summer legumes, N₂ fixation, xylem ureide, ¹⁵N natural abundance, soil health, wheat yield

INTRODUCTION

Pakistan is a country situated in south Asia between latitude 23.5 and 37°N. The grain legumes are regarded as minor crops by farmers and proper attention is not paid to their management^[1]. The nitrogen fixation by legumes was quantified for the first time through survey of summer legumes in the farmer's field in Pothwar region by Ali *et al.*^[2]. The survey result showed that majority of farmers in the Pothwar do not inoculate the summer

legumes, in contrast to inoculation of chickpea by a few farmers. Mungbean and mashbean will nodulate more readily with naturalized population of Rhizobia than soybean, which has rather specialized requirements for compatible rhizobia. In Pakistan, Rhizobium inoculation has not been used traditionally for soybean cultivation and in the absence of inoculation with highly effective Rhizobia, low N₂ fixation activity is almost inevitable. Chalk *et al.*^[3] reported that legumes not only improve soil structure but also nitrogen of soil through nitrogen

fixation they also add organic matter, increase soil nitrogen, balance soil nutrients, improve soil physical condition and break soil born disease cycle.

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 calorimetric 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^[4]. 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^[5]. 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.

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^[6].

To develop productive and sustainable legume-cereal rotation system for rainfed areas in Pothwar, a rotational field experiment at University of Arid Agriculture, Rawalpindi (UAAR), was set in 1998-99 to increase legume intensity through their interaction in summer cereal crop and to see if the xylem solute technique could substitute for natural ^{15}N abundance technique. The more specific objectives are as under:

- Comparative assessment of the quantity of N_2 fixed in legumes by xylem solute and natural ^{15}N abundance techniques.
- To improve the soil productivity through residual nitrogen fixed by legumes.
- To assess effects of summer legumes on winter wheat production.

MATERIALS AND METHODS

Rotational field experiments were undertaken at UAAR Research Farm (33°38'N, 73°04'E), in 1998-99. The climate of the region is warm temperate. Average annual rainfall ranged between 500-750 mm, with a summer dominance (70% between July and September). Meteorological data was collected at RAMC, UAAR station and is presented in Fig. 1. The legumes crop i.e. Soybean (Var. NARC 1), Mungbean, (Var. NM 92), Mashbean, (Var. NARC 2) along with maize (Var. Neelam) without and with N-fertilizer @ 100 kg N ha⁻¹ as urea were sown with seed rates 100, 20, 20 and 35 kg ha⁻¹. Three legumes species were inoculated at sowing with effective brady rhizobia. Basic plot size was 5x5 m². Experimental design was a randomized complete block with four replications. About two weeks after grain harvest of the summer crops, all plots were sown with wheat (Inqlab 91) at a rate of 0 and 100 kg N ha⁻¹ applied as urea at sowing. Phosphorus was added to all plots (both summer and winter) at sowing as single super phosphate (35 kg P ha⁻¹). Weeds were controlled by hand hoeing.

Crop data collection and soil analysis: Soil parameters measured were texture, soil bulk density, total organic carbon, particulate organic matter and soil NO₃-N. Shoot biomass and yield of each crop were recorded. Xylem sap from each legumes crop at flowering and pod fill stage were also taken.

Soil samples and analysis: A sampling tube with 4-8 cm diameter was used to collect samples to 90 cm depth. Each segment of core (0-10, 0-30, 30-60 and 60-90 cm) was weighed fresh to determine moisture content and sub samples for soil nitrate-N estimation. Bulk density was estimated by using a core sampler of known volume. TOC^[7] and POM C^[8] were also determined. Nitrate-N was determined by Salicylic Acid method^[9]. The intensity of yellow color was quantified at 410 nm for nitrate nitrogen determination.

Plant sampling and analysis: Assessment of N_2 fixation by xylem solute technique: N_2 -fixation were estimated by Xylem Solute Technique, Sap were collected at the flowering and pod-fill stage by Vacuum Extraction Method, then concentrations of ureide, nitrate and amino-N were determined by prescribed methods to calculate the relative abundance of ureide-N and % Pfix (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 (%Pfix) were estimated.

%Pfix = 1.6 (%RUN-7.7) for plants at vegetative and flowering stage of growth.

%Pfix = 1.6 (%RUN-15.9) for plants during pod fill

Calculating N fixed (kg ha^{-1}): The legume N was calculated from the measure of biomass accumulation and tissue N-content.

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

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

$$\begin{aligned} \text{Amount of } N_2 \\ \text{fixed kg ha}^{-1} &= \%Pfix \times \text{Crop N kg ha}^{-1} \times 1.5^* \end{aligned}$$

*1.5 factor were used to include an estimate for contribution by below ground $N^{[10]}$.

In crop legumes, net return of fixed N to the soil was depend upon the amount of N removed in seed. Therefore, the potential benefits of fixed N in vegetative residue of crop legumes were determined by the N-balance at final harvest.

$$\text{N-balance} = (\text{N}_2 \text{ fixed}) - (\text{shoot N} + \text{grain N})$$

Assessment of N_2 fixation by natural ^{15}N abundance technique: At estimated maximum biomass of the summer crops (late pod-fill stage in the legumes), whole shoots were harvested from a 1m^2 quadrat in each plot, oven dried at 65°C to a constant mass, then weighed, finely ground to pass through a 2 mm sieve and sent to CSIRO, Canberra where samples were analyzed for total N and $\delta^{15}\text{N}$ using an automated N and C analyzer/mass

spectrometer (ANCA-SL/20-20 stable isotope mass spectrometer, Europa Scientific, Crewe, UK). Maize samples were used as the non-fixing reference plant in N_2 -fixation calculation. Grain yield, $\delta^{15}\text{N}$ and N were determined from harvested 1m^2 quadrates. Wheat biomass and grain yield was assessed at crop maturity from two 1m^2 quadrates in each plot.

In the determination of N_2 fixation, shoot and grain ^{15}N values were expressed with reference to air N_2 as follows:

$$\delta^{15}\text{N} = 1000 (R_{\text{sample}} - R_{\text{air}}) / R_{\text{air}}$$

Where, R is the ratio mass 29/mass 28. The natural abundance of ^{15}N was expressed as $\delta^{15}\text{N}$ (parts per thousand or ‰) relative to atmospheric N_2 (0.3663 atoms % ^{15}N) as described by Shearer and Kohl^[11]. By definition, the $\delta^{15}\text{N}$ of air N_2 is zero. The proportion (Pfix) of legume N derived from N_2 fixation (Pfix) was calculated from measurement of ^{15}N abundance as

$$\text{Pfix} = 100 (x - y) / (x - z)$$

Where, x is the Delta ^{15}N of shoots of weeds deriving all of their N from the soil; y is the delta ^{15}N of soybean, mungbean and mashbean shoots; z is delta ^{15}N of soybean, mungbean and mashbean receiving all of their N from N_2 fixation. The value for z for all three species was $-2.5\text{‰}^{[10]}$.

Statistical analysis: The data collected was subjected to statistical analysis following ANOVA technique and treatment differences were differentiated using LSD test. The correlation and regression between Pfix and N_2 fixation with shoot N and yield of legumes species were also determined to standard statistical procedures^[12].

RESULTS AND DISCUSSION

Yield of summer legumes and maize: Performance of legumes varied greatly between species. Of the legumes, Soybean produced the highest (average of 7.46 t ha^{-1}) shoot dry matter and 156 kg ha^{-1} shoot N, compared with 3.4 and 4.5 t DM ha^{-1} and 56 and 89 kg N ha^{-1} for mungbean and black gram, respectively (Table 2). Maize along with N fertilizer produced largest crop (13.56 t ha^{-1}) shoot DM as compared to maize without N fertilizer (9.8 t ha^{-1}). Maize responded well to fertilizer N. Shoot biomass yield were reflecting to a large extent July-August rainfall (340-360 mm)(Fig. 1). Black gram yields were greater than those of the mungbean and soybean. Average grain yield of three species were 1.68 (Sb),

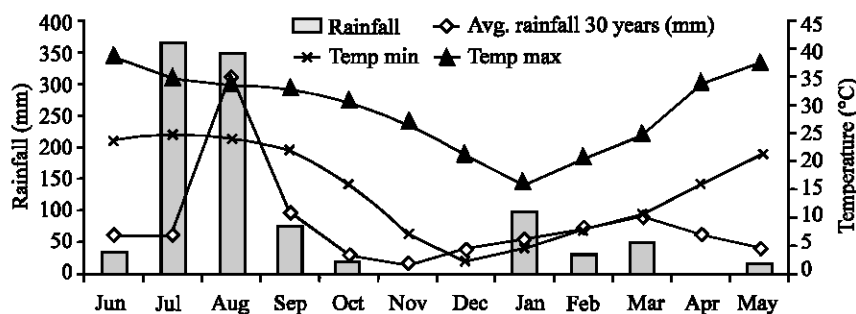


Fig. 1: Maximum, minimum temperature (°C) and comparison of actual rainfall with the average of 30 years at UAAR (June 1998-1999)

Table 1: Surface soil (0-10 cm) properties of experimental site

Texture	Silty clay loam*
Bulk density (g cm^{-3})	1.60
pH	7.20
ECe (dS m^{-1})	0.33
Organic C (%)	0.65
$\text{NO}_3\text{-N}$ ($\mu\text{g g}^{-1}$)	6.30
Olsen P (mg kg^{-1})	7.80

*Clay-28%, Silt-35%, sand-37%

0.82 (Mb) and 1.83 t ha^{-1} (Bg). Grain N data showed that soybean grain N were much greater than mungbean and black gram. Average grain N yield of soybean were 103 kg ha^{-1} followed by 66 kg ha^{-1} (Bg) and 31 kg ha^{-1} (Mb). Our findings are similar to those of Ali *et al.*^[13,14].

Legume N_2 fixation: Estimates of proportion of plant N derived from N_2 fixation (Pfix) and the amount of N_2 fixed of soybean, mung bean and black gram by xylem solute and natural ^{15}N abundance techniques are presented in Table 3 and 4. Although experimental treatments and environmental or nutritional values have generated a large range of Pfix values (0-98%) and inputs of fixed N, it appears that potential BNF for most species is in the range of $200\text{-}300 \text{ kg N ha}^{-1} \text{ crop}^{-1}$ ^[15]. However, since crop N is portioned either into seed, or vegetative parts at crop maturity, not all of the N_2 fixed will be available for return to the soil. The final contribution of fixed N to the soil following harvest will depend upon the N-balance at harvest, which is determined by the difference between the amounts of N_2 fixed and seed N removed. The influence of growing season rainfall and concentration of soil $\text{NO}_3\text{-N}$ detected at sowing (Table 1) were believed to be major factors contributing to the observed variation. There was some evidence to suggest that rainfall effects on %Pfix values for dryland crops may have been related to the growth demand for N^[16]. Because of the uncertain and erratic rainfall with summer dominance (70% between July and September) fallowing before the winter wheat for the conservation of soil water is common practice in

Pothwar region of the northern Punjab (85% of wheat crop in Pothwar may be sown after 6-7 months of fallow). Cultivation of summer legume crops therefore, constitutes an alternative to traditional cropping practices of leaving the land fallow.

Pfix and N_2 fixation at flowering and pod fill stage by xylem solute technique: The data in Table 3 shows that the mean % Pfix of soybean was higher (89) at pod fill stage followed by black gram (82) and mung bean (64). However, at flowering stage %Pfix was higher in black gram (88) than soybean (77) and mung bean (46), respectively. Present results are in line with the findings of Chiu *et al.*^[17], who reported that the percentage of N derived from N_2 fixation was 41% and N fixed was estimated at 33 kg N ha^{-1} . Peoples *et al.*^[18] indicated that the proportion of soybean N derived from N_2 fixation ranged between 4 and 96%. The data pertaining to the amount of N_2 fixed (kg ha^{-1}) shows that the soybean significantly increased the amount of N_2 fixation with maximum values of 207 kg ha^{-1} than that of mung bean (55 kg ha^{-1}) and black gram (82 kg ha^{-1}). N-balance data shows that the mean N-balance values of soybean were -52.2 followed by -74 (Bg) and -32 kg N ha^{-1} (Mb).

Pfix and N_2 -fixation by natural ^{15}N abundance technique: The natural ^{15}N abundance values for both N_2 fixing summer legumes and reference maize crop produced % Pfix estimates of 60 and 61% for soybean and black gram, respectively and 48% for mung bean. Soybean, mung bean and black gram had similar (42-64%) values of grain and plant ^{15}N (Table 4). % Pfix values were similar to those from farmer field experiments in Pothwar (range 31-64%)^[14] and the Hill and Terai region of Nepal (mean of 62%)^[19]. Mung bean and black gram % Pfix values were also similar to those from farmer crops surveys of the same species in the North West Frontier Province of Pakistan (mean of 47%)^[20] and those from a local Pothwar survey of 12 mung bean and black gram crops (range 45-90%, mean of 77%)^[2].

Table 2: Shoot N, DM, grain N and grain yield data of Soy bean (Sb), Mung bean (Mb), Black gram (Bg) and Maize (Mz)

Treatments	Shoot N (%)	Shoot DM (t ha ⁻¹)	Shoot N ^a (kg ha ⁻¹)	Grain N (%)	Grain yield (t ha ⁻¹)	Grain N ^b (kg ha ⁻¹)
Sb	2.14±0.19	7.46±0.56	156.00±4.02	6.14±0.13	1.68±0.13	103.00±7.33
Mb	1.68±0.04	3.39±0.34	56.25±5.79	3.70±0.05	0.82±0.04	30.75±3.88
Bg	1.98±0.22	4.52±0.46	89.5±13.82	3.64±0.04	1.83±0.24	66.50±7.89
Mz0N	0.82±0.04	9.76±0.80	80.25±10.09	1.47±0.05	4.35±0.34	64.25±6.02
Mz+N	0.91±0.66	13.56±0.66	123.3±7.04	1.63±0.10	5.86±0.47	96.25±8.62
CV (%)	20.00	13.18	15.30	5.06	18.83	15.93
LSD(0.05)	0.46	0.84	23.81	0.257	0.843	17.70

^a(shoot dry matter kg ha⁻¹) × (shoot %N)

^b(grain yield kg ha⁻¹) × (grain %N)

Table 3: N₂-fixed by Soy bean (Sb), Mung bean (Mb) and Black gram (Bg) using xylem solute technique

Treatments	Pfix at pod fill stage (%)	^a N ₂ -fixed (kg ha ⁻¹)	^c N-balance (kg ha ⁻¹)
Sb	88.59±3.71	206.8±9.53	-52.2±10.76
Mb	64.56±3.60	55.00±7.31	-32.0±4.52
Bg	82.28±4.15	82.00±10.65	-74.0±4.37
Mz0N			
Mz+N			
CV (%)	10.93	18.15	29.95
LSD (0.05)	14.84	35.97	25.72

^a(%Pfix at pod fill stage) × (shoot N kg ha⁻¹) × 1.5

^c(N₂ fixed kg ha⁻¹)-(shoot N kg ha⁻¹-grain N kg ha⁻¹)

Table 4: N₂-fixed by Soy bean (Sb), Mung bean (Mb) and Black gram (Bg) using Natural ¹⁵N abundance technique

Treatments	δ ¹⁵ N Shoot (‰)	δ ¹⁵ N Grain (‰)	Pfix of plant ¹⁵ N (%)	^a N ₂ -fixed (kg ha ⁻¹)	^c N-balance (kg ha ⁻¹)
Sb	-0.36±0.18	-0.63±0.13	60.03±3.19	145.3±9.29	-113.7±5.76
Mb	0.45±0.28	0.50±0.22	48.22±5.23	41.25±7.18	-45.7±4.70
Bg	-0.30±0.20	-0.18±0.25	61.05±3.56	80.00±8.97	-76.0±9.06
Mz0N	3.15±0.24	2.72±0.23			
Mz+N	4.24±0.30	3.19±0.34			
CV (%)	34.71	34.95	5.58	19.95	
LSD (0.05)	0.767	0.604	5.51	30.72	

^a(%Pfix of plant ¹⁵N) × (shoot N kg ha⁻¹) × 1.5

^b(%Pfix) × (shoot N kg ha⁻¹)

^c(N₂ fixed kg ha⁻¹)-(shoot N kg ha⁻¹-grain N kg ha⁻¹)

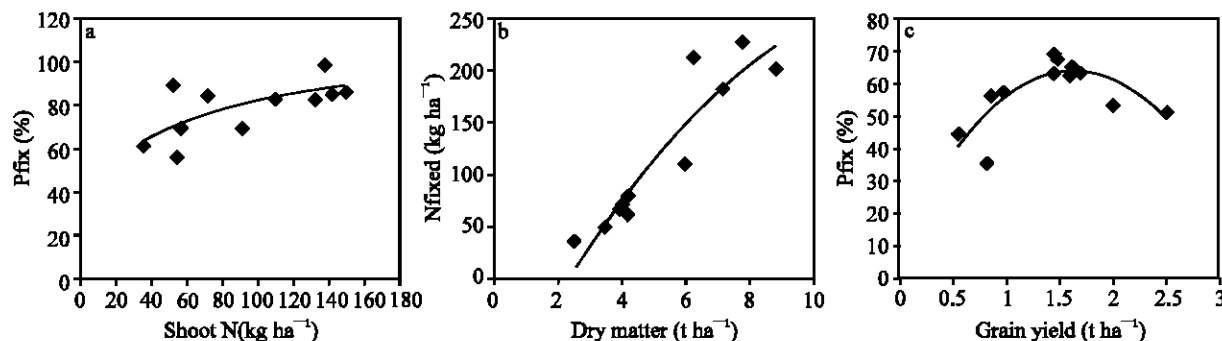


Fig. 2: Relationship between (a) shoot N and Pfix (b) dry matter and N-fixed © grain yield and Pfix using xylem solute technique. Individual points are mean values of four replicates. Regression functions are as follows:

(a) $Y = 17.285X + 0.6039$ ($r^2 = 0.4491$; $p < 0.05$)

(b) $Y = -2.009X^2 + 56.174X - 113.23$ ($r^2 = 0.8748$; $p < 0.05$)

(c) $Y = -19.158X^2 + 63.853X + 10.28$ ($r^2 = 0.6348$; $p < 0.05$)

The total amount of N fixed is the resultant of two characteristics: proportion of plant N fixed (Pfix) and total amount of N accumulated in the crop. N₂ fixation capacity was different for different species. Nitrogen fixation of δ¹⁵N samples indicated that the soybean fixed the higher nitrogen (145 kg ha⁻¹) than that of mung bean (41 kg ha⁻¹) and black gram (80 kg ha⁻¹). Soybean, mung bean and black gram provided 76 to 114 kg N ha⁻¹ for the succeeding wheat crop (Table 4). Comparison of N₂ fixation and amounts of seed N harvested

have been under taken for many crops and examples of fixed N-balance range from as little as -132 kg N ha⁻¹ to as much as +80 kg N ha⁻¹ in soybean, -34 to 64 in ground nut, -42 to +34 kg N ha⁻¹ in chick pea, -11 to +25 in lentil, -32 to 96 in pea and -42 to 135 kg N ka⁻¹ in lupin^[15], depending upon the amounts of N₂ fixed, the harvest index for N (proportion of total crop N removed in seed) and weather the vegetative residues are removed from the field^[21,22].

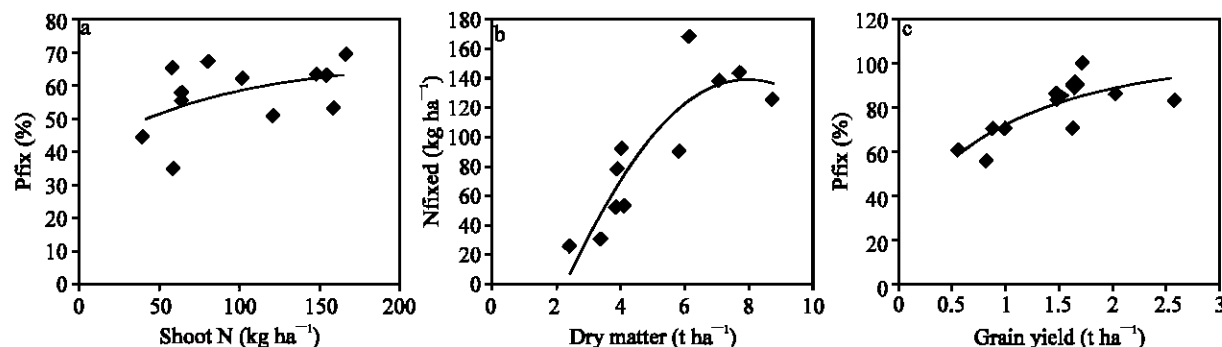


Fig. 3: Relationship between (a) shoot N and Pfix (b) dry matter and N-fixed © grain yield and Pfix using natural ^{15}N abundance technique. Individual points are mean values of four replicates. Regression functions are as follows:

- (a) $Y = 9.3047X + 15.18$ ($r^2 = 0.2138$; $p < 0.05$)
 (b) $Y = -4.3178X^2 + 69.081X - 136.25$ ($r^2 = 0.8023$; $p < 0.05$)
 (c) $Y = 22.796X + 71.883$ ($r^2 = 0.5918$; $p < 0.05$)

Relationship between shoots N, dry matter and grain yield with legumes N_2 fixation:

Simple regression function for Pfix and N_2 fixation with shoot N, dry matter and grain yield are presented in Fig. 2 and 3. Pfix was strongly correlated with grain yield ($r^2 = 0.63$; $p < 0.05$) and ($r^2 = 0.59$; $p < 0.05$) than shoot N ($r^2 = 0.45$; $p < 0.05$) and ($r^2 = 0.21$; $p < 0.05$). With the legume dry matter- N_2 fixation relationship, dry matter was more strongly correlated with N_2 fixed of xylem ureide ($r^2 = 0.87$; $p < 0.05$) as compared with $\delta^{15}\text{N}$ ($r^2 = 0.80$; $p < 0.05$). Herridge *et al.*^[6] elucidated that only soil nitrate and grain yield, but not shoot N, could be used as independent variables for regression analysis against total N_2 fixed because shoot N was used to calculate total N_2 fixed (i.e. shoot $\text{N} \times 1.5 \times \text{Pfix}$) and would be auto-correlated. The regression for total N_2 fixed was not as strong as for Pfix because of variation in the relationship between grain yield and crop N associated with growth and environmental effects as well as the difficulties in sampling for maximum biomass.

Effect of summer legumes on soil health: The result obtained on soil quality indicators like $\text{NO}_3\text{-N}$, TOC and POM C at summer harvest upto 0.9 m are presented in Table 5.

Soil $\text{NO}_3\text{-N}$: Residual (post summer) soil nitrate-N derived between 3.72 and 10.31 kg ha^{-1} for legumes and 4.47 and 10.9 kg ha^{-1} for maize (Table 5). Average values at surface soil (0-10 cm) after the harvest of Sb, Mb, Bg were 8.66, 9.16 and 9.12 kg ha^{-1} , respectively and 10.9 and 8.82 kg ha^{-1} for maize without and with N, respectively. The mean maximum $\text{NO}_3\text{-N}$ concentration of 10.31 kg ha^{-1} was found at 0-30 cm depth in the plot

Table 5: Effect of summer legumes on soil quality indicators

Treatments	0-10 cm	0-30 cm	30-60 cm	60-90 cm
$\text{NO}_3\text{-N kg ha}^{-1}$				
Sb	8.66±0.42	10.31±0.79	6.31±0.80	3.71±0.08
Mb	9.16±0.40	10.19±1.25	6.55±0.06	4.45±0.14
Bg	9.12±0.43	10.15±0.46	7.22±0.47	4.37±0.06
Mz0N	10.89±0.85	9.56±0.77	6.48±0.42	4.79±0.07
Mz+N	8.81±0.36	9.58±0.45	6.6±0.41	4.47±0.08
CV (%)	11.18			
LSD (0.05)	1.198			
TOC t ha⁻¹				
Sb	8.65±2.54	10.31±0.48	6.31±0.67	3.72±0.17
Mb	9.15±2.68	10.18±0.47	6.51±0.23	4.35±0.25
Bg	9.12±2.67	10.11±0.28	7.21±0.27	4.41±0.29
Mz0N	10.90±0.31	9.49±0.41	6.48±0.54	4.66±0.47
Mz+N	8.82±2.61	9.59±0.29	6.61±0.43	4.47±0.54
CV (%)	33			
LSD (0.05)	1.578			
POM C t ha⁻¹				
Sb	1.67±0.12	0.96±0.07	0.46±0.04	0.40±0.02
Mb	1.41±0.09	1.1±0.10	0.65±0.04	0.41±0.04
Bg	1.61±0.08	0.79±0.09	0.40±0.13	0.50±0.04
Mz0N	1.47±0.09	0.76±0.27	0.54±0.03	0.47±0.04
Mz+N	1.57±0.09	0.91±0.06	0.60±0.06	0.44±0.03
CV (%)	22.33			
LSD (0.05)	0.273			

Table 6: Prior crop Soy bean (Sb), Mung bean (Mb), Black gram (Bg) and Maize (Mz) and N fertilizer effects on wheat dry matter and grain yield

Crop sequence	Shoot DM (t ha ⁻¹)	Grain yield (t ha ⁻¹)
Sb/W	12.15±0.41	3.85±0.18
Sb/w+ 100N	15.25±0.33	4.04±0.28
Mb/W	12.28±0.48	4.10±0.30
Mb/w+ 100N	17.20±0.45	5.37±0.24
Bg/W	11.63±0.49	3.16±0.13
Bg/w+ 100N	14.85±0.46	4.17±0.25
Mz0N/W	11.85±0.61	3.60±0.10
Mz0N/w+ 100N	15.33±0.57	4.50±0.13
Mz+N/W	16.20±0.38	4.60±0.09
Mz+N/w+ 100N	16.75±0.43	5.10±0.17
C.V (%)	5.83	8.81
LSD (0.05)	1.26	0.56

previously under soybean followed by 10.18 kg ha^{-1} at the same depth in the plot previously under mungbean. Estimation of the distribution of residual Nitrate-N in the soil profile indicated decreasing trend towards profile depth. However, most of the nitrate remained in the top 30 cm. The Co-efficient of Variation (CV) of $\text{NO}_3\text{-N}$ in the soil profile was 11.18%. Marcellos *et al.*^[23], observed similar trend of $\text{NO}_3\text{-N}$ distribution in the soil profile. Herridge *et al.*^[24] has reported that the measured effect of annual legumes on the soil fertility is through effects on plant available nitrate nitrogen through experiment. He also concluded that nitrate nitrogen in the plots following legumes were an average 30 kg ha^{-1} greater than the levels in the cereal plots.

Soil TOC: TOC ranged from $3.72\text{-}10.31 \text{ t ha}^{-1}$ at different depth in the plots where soybean crop was harvested, followed by $4.35 \text{ to } 10.18 \text{ t ha}^{-1}$ in the plot of mung bean. Maximum TOC (10.31 t ha^{-1}) was found at the depth of 0-30 cm in the soybean plot followed by mung bean (10.18 t ha^{-1}) and black gram (10.11 t ha^{-1}), respectively (Table 5). Distribution of residual TOC in the soil profile showed that TOC contents were more at surface soil (0-30 cm), where as TOC concentration was lower at deeper depth. C.V of TOC at different depth were 33%. When compared with the initial level, a non-ignorable increase was observed in soil TOC by the end of summer harvest. Thus beside a very much contribution, legumes has contributed to soil health^[25].

POM C: The results obtained on POM C in the soil profile showed almost similar trend at different depth when compared to TOC. It ranged from $0.40 \text{ to } 1.67 \text{ t ha}^{-1}$ with C.V of 22.33%. It is revealed from the results that maximum POM C remained in the surface soil (0-10 cm). Replenishment of soil organic matter is the corner stone to regenerating soil health, therefore, a physically-separated pool of the soil organic matter, the Particulate Organic Matter (POM) should reflect the balance between organic (residue) inputs, persistence and decomposition as determined by the soil environment. As an indicator of soil health, POM should be sensitive enough to allow changes in management practices to detected with in a few growing seasons and as changes with in growing season are not large^[8].

Yield of following wheat: For 0N wheat, shoot DM and grain yields were more in the plots previously under legumes than following the 0N maize (Table 6). Total biomass yield in plots without N under soybean, mung

bean, black gram and maize0N and maize+N was 12.1, 12.3, 11.6, 11.8 and 16.2 t ha^{-1} , respectively. Similarly highest grain yield was 4.1 t ha^{-1} in the plot with out N under mung bean followed by previous soybean, maize0N and black gram crops ($3.8, 3.6, 3.2 \text{ t ha}^{-1}$, respectively). Increase was 2.5 and 3.6% for DM when the prior crops were soybean and mung bean, respectively and 7 and 14% for grain yield in the same plots. However, contribution of residual effect of black gram was not significant. The wheat +N grown after mung bean produced the highest shoot DM and grain yield (17 and 5.4 t ha^{-1} , respectively)(Table 6). Wheat shoot DM increased by an average of 24% (N rates of 100 kg ha^{-1}). Grain yield responded similar with average increase of 20%. Our results are in line with those of Ali *et al.*^[14] and Ali *et al.*^[13]. Herridge *et al.*^[24] has reported that the measured effect of annual legumes on the soil fertility is through effects on plant available nitrate nitrogen through experiment. He also concluded that nitrate nitrogen in the plots following legumes were an average 30 kg ha^{-1} greater than the levels in the cereal plots. Singh and Verma^[26] found that wheat yield was significantly higher after cowpea, mung bean and mash bean than that obtained after pigeon pea, fallow and pearl millet. Ali *et al.*^[14] concluded that there were residual benefits for the wheat of the fertilizer N applied to the previous maize crop, which were similar in magnitude to the legume benefits and when the wheat was fertilized with N, the legume benefits disappeared. Responses to fertilizer N, irrespective of prior crop were large, which reflects that soil was deficient in plant available N.

For reliable measures of N_2 fixation it is suggested to utilize more than one methodology in field N_2 fixation studies wherever possible. In spite of the constraints, the N_2 fixed by natural ^{15}N abundance technique gave reasonable consistent results because this technique is more precise, it requires a precise mass spectrometer and meticulous analytical procedures. Technically the field-sampling of xylem contents is simple and the analysis of N-components (i.e. ureide, α -amino N and nitrate) can be done by calorimetric assays, therefore research is needed to strengthen the cost effective xylem ureide technique as a reliable routine laboratory analysis in developing countries like Pakistan.

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