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Effect of Fertilizer, Inoculation and Sowing Time on Free Amino Acids and Mineral Nitrogen Content of Field Grown Mature Soybean Seeds

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Abstract: The study was conducted on two different sowing dates in response to various level of applied N fertilizer on free amino acids and mineral N content (viz., NO_3-N, NH_4-N and NO_2-N) on mature soybean CV.Williams-82 seeds. Fertilizer treatments were applied to both non-inoculated (non-inoc) and inoculated (inoc) field crop. Results showed that fertilizer treatments in general significantly ($P<0.05$) reduced the free amino acids and NH_4-N of both sowing (BS) as well as the NO_3-N of early sowing (ES) and NO_2-N of late sowing (LS) seeds, but insignificant influence was recorded in NO_3-N of LS seeds. While NO_2-N showed a significant response in ES seeds. A maximum significant level of free amino acids (0.207-0.223 mg g^{-1}) and NH_4-N (0.9520-1.1448 g kg^{-1}) was noted in T_2, while a maximum level of NO_3-N (0.0793-0.0867 μM) was noted in T_3 of BS field seeds respectively. However, a significant level of NO_2-N was recorded in T_1 (3.950 g kg^{-1}) of LS followed by T_5 (1.968 g kg^{-1}) of ES seeds. Results further suggested that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation in general significantly and positively influenced the NO_3-N and NO_2-N, but decreased the NH_4-N of BS field seeds, while the free amino acids were generally increased in LS, but non-significantly respond in ES seeds. A maximum level of free amino acids was recorded in T_2 inoc (6.750-3.974 g kg^{-1}), NO_3-N in T_4 and T_3 inoc (0.1057 and 0.1950 μM) and NO_2-N in T_6 inoc (6.750-3.974 g kg^{-1}), while a maximum level of NH_4-N is noted in T_2 inoc (0.9013-0.9780 g kg^{-1}) of BS seeds, respectively. Therefore, it can be safely concluded that low level of mineral N content in ES than LS seeds might be due to their rapid incorporation into various nitrogenous organic compounds during the entire course of seed development to maturity. The correlation coefficient (r) studies revealed that free amino acids exhibited significant positive association with NO_2-N (0.426), soluble protein (0.824) and oil content (0.618) of LS seeds. While they showed significant positive correlation with NO_3-N (0.403), NH_4-N (0.454) and soluble sugars (0.601), but negative with grain yield of LS seed.

Key words: Soybean, fertilizer, inoculation, sowing time, free amino acids, mineral nitrogen content

Introduction

Amino acids are small, water-soluble organic compounds ubiquitous in living organisms. There are 22 commonly occurring amino acids, making up the backbone of proteins are also found independent of protein free in the cytoplasm. Some exists as components of non-proteinaceous molecules and many are important for intermediary metabolism. In plants there have been over 100 non-protein amino acids isolated and identified. Most of them are known to have a function in nitrogen metabolism (Ting, 1982). Most of the calories in human-diet comes from seed protein of cereals. Legume seeds viz., beans, peas and soybean make a smaller but still an important contribution. Soybeans are usually rich, fairly well balanced protein source; about 40% of their dry weight is protein compared to about 12% for most cereal grains (Salisbury and Ross, 1992). Amino acids are one of the most important products of photosynthesis and their synthesis take place in most of the cell components (Illahi, 1996).

The elemental composition of food grains is important in human and animal nutrition. A great deal of work has been done and is known about the elemental composition of food grains consumed by humans, but much less is known about the genetic and environmental factors controlling their composition. A very little is known about the mineral nitrogen (NO_3-N, NH_4-N and NO_2-N) status of mature soybean seeds in response to various levels of added fertilizer (with and without inoculation) and sowing time. Recent investigations indicated that soybean not only contain appreciable amount of protein, oil and carbohydrates to some extent, but also has a potential to become both marketable human food grain and an important poultry feed.

Research revealed that the N concentration of soybean and rice grain increased with increase in N dose, respectively (Lathwell and Evans, 1951; Singandhupe and Rajput, 1990). They also found that N concentration of soybean seed ranges between 1.13-1.21% and positively correlated with grain yield. Liu and Hadley (1971) concluded that NO_3 content was higher in nodulating than non-nodulating lines of soybean. A significant positive correlation was also observed between NO_3 content and seed protein. Hanway and Weber (1971 a,b) found that N in nodulating and non-nodulating soybean plant seeds at maturity influenced by added N fertilizer.

Inoculation alone and in combination with N, P and K fertilizers also influenced the accumulation of N, P and K status of various plant parts of soybean. Liang *et al.* (1991) reported that N accumulation in all organs of soybean plants was 15.5 and 13.2% higher in the inoculated plants than those given N fertilizers. Several others have also got higher seed N contents in inoculated than non-inoculated controls (Bergerson *et al.*, 1992; Ghobrial *et al.*, 1995). They pointed out that inoculation and nodulation had increased seed total N content by 75-90% as compared with non-inoculated soybean, respectively.

The present study was, therefore designed to evaluate the beneficial effect of added N fertilizer (with and without inoculation), sowing time on the free amino acid and mineral N contents of mature soybean seeds. The study was also initiated to furnish the information on the nature of association among different chemical components and grain yield.

Materials and Methods

Two-year field experiments on soybean *Glycine max* L. Merill CV.Williams-82 at two different sowing dates were carried out during the 1st week of July, 1996 (late planting) and June 1997, (early planting) in Agricultural Research Institute (ARI), Quetta, respectively. Seven different treatments (T) of fertilizer were applied to both non-inoculated (non-inoc) and inoculated (inoc) set of experiments. T_1 was kept control; T_2 contained 60+30 P_2O_5, K_2O kg ha^{-1} and from T_3 to T_7 N fertilizer was added @ 25, 50, 75, 100 and 125 kg ha^{-1} along with combination of the same constant dose of P and K, respectively. The source, time and methods of fertilizer application already been explained by Achakzai *et al.* (2002a). The seeds of each treatment were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown colouration. Finally, the seeds of each treatment were ground in a grinder, sieved through Mesh No. 60 (Johnson and Firth Brown Ltd., London). Air-dried defatted soybean seed powder (0.2 g) from each sample was homogenized in 20.0 ml of phosphate buffer solution (0.1 M, pH 7.0) at room temperature for 16 h, with continuous shaking at 300 rpm (Edmond BÜhler 7400 TÜbingen). The sample was then centrifuged at 5,000 rpm using IEC B-20A Centrifuge (Damon/IEC Division) for 20 min, filtered through Whatmann filter paper, stored at 4°C and then used for the determination of the following chemical components:

Free amino acids (mg g^{-1}): Total free amino acids were spectrophotometrically determined following the procedure described by Hamilaton and Van Slyke (1943). Air-dried, defatted and buffer extracted sample (1.0 ml) was placed in a test tube, mixed in a mixture of 1.0 ml ninhydrin (2% m v^{-1}) and 1.0 ml of pyridine solution (10% v/v). The test tube was heated for 30 min on a boiling water bath. The contents of the tube was diluted with 50 ml water and the absorbance of the solution was measured at 570 nm using Spectrophotometer (Hitachi U-1100, Japan). Total free amino acids were then calculated: (Equation 1).

Nitrate determination (μM): Nitrate was determined spectrophotometrically by following the procedure of Cataldo *et al.* (1975). An aliquot of 0.5 ml buffer extracted sample and 0.2 ml salicylic acid (5% w v^{-1} in conc. sulfuric acid) was added in a test tube. After 20 min 4.3 ml of NaOH (2.0 M) was added in it and the contents of the tube were thoroughly mixed. The absorbance was monitored at 410 nm using Spectrophotometer. A series of 5 different standard solutions (0.125 μM-1.00 μM) from the stock solution of potassium nitrate (25.0 μM) were prepared. A calibration graph of these standard solutions was drawn and used for the calculation of NO_3-N in the sample.

Ammonium determination (g kg^{-1}): Ammonium ion was determined by spectrophotometric method as advised by Qiu *et al.* (1987). An aliquot of 1.0 ml sample was mixed with 0.5 ml of salicylic acid-sodium citrate solution, 0.5 ml of sodium nitroprusside solution (1.0% w v^{-1}) and 0.05 ml of NaOCl solution simultaneously in a test tube. The solution was mixed well and diluted with 3.4 ml of water. After 1 h the absorbance was measured at 697 nm against a reagent blank prepared in the same manner but without having ammonical nitrogen. A series of 5 different standard solutions (0.1 - 0.5 mM) from the stock solution of NH_4Cl (0.01 M) were prepared. A calibration graph of the standard solutions was drawn and used for calculated of NH_4-N in the sample.

Nitrite determination (g kg^{-1}): Nitrite was also spectrophotometrically determined following the procedure described by Flamerz and Bashir (1981). An aliquot of the sample (0.5 ml) was taken in a test tube containing 0.5 ml of 4-aminosalicylic acid solution (0.25%

Equation 1: Free amino acids ($mg\ g^{-1}$) =

$$\frac{\text{Abs. of sample X vol. of sample X dilution factor X 1000}}{\text{Weight of the sample}}$$

w v^{-1}) and 0.5 ml naphtha-1-ol solution (0.35% w v^{-}).[1] Diluted the mixture to 5.0 ml with water and mixed thoroughly. The absorbance was monitored at 520 nm against a reagent blank (phosphate buffer). A series of 5 different standard solutions (5-25 ppm) from the stock solution of sodium nitrite (1000 ppm) were prepared. A calibrated graph of the standard solutions was drawn and used for the calculation of NO_2-N in the sample.

The data obtained were statistically calculated by following the procedure described by Steel and Torrie (1980). MSTAT-C Computer software package for statistical analysis was used for calculation of analysis of variance (ANOVA) and least significant difference test (LSD). Simple correlation coefficient (r) studies were also worked out for all mentioned nutrients as well as other chemical components and grain yield in mature soybean seeds, which has been already explained by Achakzai and Kayani (2002a) and Achakzai *et al.* (2002).

Results and Discussion

Data presented in Table 1 showed that in response to different level of added fertilizer with and without inoculation and its interaction exhibited highly significant ($P<0.01$) effect on the free amino acid and mineral N contents of mature soybean seeds of both sowings (BS).

Free amino acids: Data presented in Table 2 showed that except of T_2, fertilizer treatments significantly but adversely affected the free amino acids of early sowings (ES), while non-significant effect (except T_2) was found in case of late sowings (LS) when compared with treatment not receiving fertilizer (T_1). Results suggested that added P and K fertilizer alone significantly increased the free amino acid levels, but generally decreased in combination with various doses of added urea fertilizer. Though, no extensive work has been done on the effect of various level of added N fertilizer on the free amino acid content of mature soybean seeds. However, few workers stated that added N fertilizer resulted reduction in the contents of some amino acids in developing seeds and also led to faster accumulation of oil content in mature soybean seeds. Therefore, present findings of ES are in confirmation with the results obtained by Ham *et al.* (1975), Sugimoto *et al.* (1998) and Achakzai and Kayani (2002b), while LS results are also in agreement with the results explained by few others (Babich and Petrichenko, 1992ab; Sugimoto *et al.*, 1997). This reduction in ES may be due to their incorporation into various nitrogenous organic compounds. Results also showed that by comparing the inoc with non-inoc in particular doses of fertilizer, inoc in general significantly and positively affected the free amino acids of LS and non-significantly influenced in ES seeds (except T_3, T_5 and T_6). Mathematically and statistically, a maximum level of free amino acids are found in T_2 inoc and non-inoc (0.232 mg g^{-1}) of BS seeds. Whereas on the basis of marginal mean values, the inoc effects are noted as 17.42 and 21.62% greater in both sowings (BS) when compared with their respective non-inoc treatments. Though nodules were not established in any set of experiment, but this silent positive effect was also noted on other chemical and agronomical parameters of the same set of experiment (Achakzai and Kayani, 2002a; Achakzai *et al.,* 2002a) as well as on the free amino acids of the pot culture experiment (Achakzai and Kayani, 2002b). However, on the basis of grand mean values, the ES seed produced 14.69% greater free amino acid content than that of LS field seeds.

NO_3-N: Data (Table 2) showed that fertilizer treatments in general significantly but adversely affected the NO_3-N content of ES field seeds (except T_2 and T_3) when compared with treatment not receiving fertilizer (T_1). Whereas non-significant effect was observed in LS field seeds (except T_6). Statistically and mathematically, a maximum level of NO_3-N is noted in T_3 (0.1013 - 0.1950 μM) dose of both year field seeds. Beside protein, oil and sugars, the elemental composition of food grain is also important in human and animal nutrition. Though a good deal of work has been done on the effects of applied fertilizer on the total N budget of soybean seeds, but very little is known about the NO_3-N status of mature soybean seeds. Most of the researchers recorded an increased level of NO_3 contents in response to various level of added N fertilizer by the developing soybean seeds. While reverse is found in the present studies and are not in agreement with the results obtained by Singandhupe and Rajput (1990), Ifsan (1991) and Pandey *et al.* (1995), but are in agreement with the Pot culture studies of Achakzai and Kayani (2002b). It is possible that in developing seeds the NO_3 source of N in response to added fertilizer was increasingly incorporated into proteins and other nitrogenous organic compounds during the course of maturity of seeds. Present study also revealed that by comparing the inoc with non-inoc

Table 1: Analysis of variance (ANOVA) for free amino acids and mineral N content of field grown mature soybean seeds in relation to added fertilizer treatments alone (A) and in combination with inoculum (B)

	Mean square for fertilizer (A)		Mean square for inoculum (B)		F-value of variables at an error of 26					
					Fertilizer (A) (Df = 6)		Inoculum (B) (DF = 1)		A X B (Df = 6)	
Variables	LS	ES	LS	ES	LS	ES	LS	ES	LS	ES
Free amino acid (mg g^{-1})	0.007	0.009	0.005	0.011	8931.2*	24389.8*	6487.9*	28328.7*	216.74*	4995.9*
NO_3-N (μM)	0.001	0.004	0.006	0.014	2043.59*	1603.33*	9197.84*	6283.7*	294.86*	750.95*
NH_4-N (g kg^{-1})	0.129	0.057	0.150	0.116	1320.69*	1849.26*	1532.46*	3735.31*	1348.89*	1060.39*
NO_2-N (g kg^{-1})	5.339	1.455	86.849	13.116	53394.0*	7824.4*	868559.0*	70557.3*	28246.0*	5096.7*

*Significant at 1% level of probability Df = degree of freedom LS = late sowing ES = early sowing

Table 2: Effect of various level of added fertilizer on the free amino acids and mineral N composition of field grown non-inoculated and inoculated mature soybean seeds

	Free amino acid (mg g^{-1})		NO_3-N (μ M)		NH_4-N (g kg^{-1})		NO_2-N (g kg^{-1})	
Treatments	LS	ES	LS	ES	LS	ES	LS	ES
T_1 (non-inoc)	0.145c	0.208b	0.0760cde	0.08233de	0.8450c	1.6300b	3.950g	1.503j
(inoc)	0.154c	0.209b	0.08867b	0.11070b	0.2057j	0.7550j	6.360b	3.901b
T_2 (non-inoc)	0.207b	0.223a	0.0770cde	0.07867ef	0.9520a	1.1448a	1.232j	1.655i
(inoc)	0.232a	0.232a	0.08567bc	0.08467cde	0.9013b	0.9780d	5.560c	2.610d
T_3 (non-inoc)	0.112e	0.109g	0.0793bcd	0.08667cde	0.9100b	0.8153g	1.106l	1.501j
(inoc)	0.120e	0.203b	0.10130a	0.19500a	0.6667g	0.8017h	3.647e	1.636i
T_4 (non-inoc)	0.115e	0.128f	0.0706def	0.04867i	0.7097ef	0.8587e	1.165k	1.755h
(inoc)	0.148c	0.135ef	0.10570a	0.09400c	0.4447h	0.7660i	3.974d	2.510e
T_5 (non-inoc)	0.113e	0.128f	0.0693def	0.06467gh	0.6560g	0.9943c	0.827n	1.968g
(inoc)	0.145cd	0.134ef	0.10270a	0.088cde	0.7233e	0.8467f	6.750a	3.974a
T_6 (non-inoc)	0.116e	0.139e	0.0320g	0.05933h	0.4160i	0.9893c	1.633i	1.345k
(inoc)	0.149c	0.184c	0.06467f	0.08467cde	0.7047f	0.8230g	3.268f	2.172f
T_7 (non-inoc)	0.115e	0.101g	0.0686ef	0.07067fg	0.7713d	0.6927k	1.069m	1.950g
(inoc)	0.134d	0.162d	0.08467bc	0.09133cd	0.7767d	0.8560ef	2.176h	2.667c
LSD (5%)	0.01061	0.01061	0.01061	0.01061	0.01678	0.01061	0.01678	0.02374
LSD (1%)	0.01435	0.01435	0.01435	0.01435	0.02269	0.01435	0.02269	0.03209
MM (non-inoc)	0.132	0.148	0.06757	0.07014	0.7514	0.9369	1.4467	1.6681
MM (inoc)	0.155	0.180	0.09048	0.10691	0.6319	0.8323	4.3227	2.7857
Grand mean	0.143	0.164	0.079	0.089	0.692	0.885	2.885	2.227
CV (%)	0.64	0.37	0.98	1.70	1.43	0.63	0.35	0.61

Mean values followed by the same letter(s) in a column are not significantly different from each other at 5% level of significance according to least significant difference (LSD) test. MM= marginal mean, non-inoc= non-inoculated and inoc=inoculated. CV= cofficient of variation. LS= late sowing and ES= early sowing. T_1= 0-0-0 kg NPK ha^{-1}; T_2= 0-60-30 kg NPK ha^{-1}; T_3= 25-60-30 kg NPK ha^{-1}; T_4= 50-60-30 kg NPK ha^{-1}; T_5= 75-60-30 kg NPK ha^{-1}; T_6= 100-60-30 kg NPK ha^{-1}; T_7= 125-60-30 kg NPK ha^{-1}

Table 3: Correlation coefficient (r) studies of free amino acid and mineral N contents also with other chemical components and grain yield of field grown mature soybean seeds in response to various level of added N fertilizer (with and without inoculation)

Variables	1 LS ES	2 LS ES	3 LS ES	4 LS ES	5 LS ES	6 LS ES	7 LS ES	8 LS ES	9 LS ES
1 LS	1.000								
ES	1.000								
2 LS	0.244NS	1.000							
ES	0.403*	1.000							
3 LS	0.285NS	0.039NS	1.000						
ES	0.454*	-0.298NS	1.000						
4 LS	0.426*	0.592**	-0.365NS	1.000					
ES	0.120NS	0.111NS	-0.369NS	1.000					
5 LS	0.824**	-0.041NS	0.216NS	0.293NS	1.000				
ES	-0.165NS	-0.342NS	0.078NS	-0.039NS	1.000				
6 LS	0.618**	0.347NS	0.074NS	0.382*	0.501**	1.000			
ES	-0.200NS	0.215NS	-0.030NS	-0.257NS	-0.279NS	1.000			
7 LS	-0.087NS	0.168NS	0.155NS	-0.283NS	-0.507**	-0.064NS	1.000		
ES	0.601**	0.595**	0.044NS	0.430*	0.042NS	-0.063NS	1.000		
8 LS	-0.303NS	-0.724**	-0.228NS	-0.494**	-0.507**	-0.272NS	0.242	1.000	
ES	0.080NS	0.339NS	-0.241NS	0.454*	0.273NS	-0.679**	0.464*	1.000	
9 LS	-0.122NS	0.020NS	-0.183NS	0.118NS	0.138NS	-0.199NS	-0.108NS	-0.099NS	1.000
ES	-0.599**	-0.296NS	-0.288NS	-0.128NS	0.683**	-0.247NS	-0.211NS	0.331NS	1.000

*And** significant at P< 0.05 and P< 0.01 respectively, while NS stands for non-significant at both level of probability.
LS= late sowing and ES= early sowing. (1) Free amino acids, mg g^{-1}. (2) NO_3-N, μM. (3) NH_4-N, g kg^{-1}. (4) NO_2-N, g kg^{-1}. (5) Soluble protein, g kg^{-1}. (6) Oil contents, g kg^{-1}. (7) Soluble sugars, g kg^{-1}. (8) Starch-content, g kg^{-1}. (9) Yield $Plot^{-1}$, g.

treatments in particular doses of fertilizer, inoculation significantly and positively influenced the NO_3-N status of both year field seeds (except T_2). Statistically and mathematically, a maximum level is noted in T_3 (0.1950 μM) of ES and T_4 (0.1057 μM) of LS seeds respectively, whereas on the basis of marginal mean values the inoculation effect is recorded as 33.91 and 52.42% greater in both year field experiments when compared with their non-inoc treatments respectively. Most of the researchers have also reported an increase in total N yield of inoc with non-inoc seeds. The same is the case in present studies and are strongly in support of the findings obtained by Crozat *et al.* (1992), Ghobrial *et al.* (1995) and Achakzai and Kayani (2002b). However, on the basis of grand mean values, the ES field seed produced 12.66% greater NO_3-N over LS field seeds.

NH_4-N: Data regarding mean values (Table 2) showed that fertilizer treatments in general significantly but adversely and non-consistently affected the NH_4-N of LS (except T_2 and T_3) and ES (except T_2) field seeds when compared with their respective control treatment (T_1). Statistically maximum significant level is recorded in T_2 (0.9520 – 1.1448 g kg^{-1}) of both year field seeds, respectively. Some workers (Singandhupe and Rajput, 1990; Ifsan, 1991; Pandey *et al.,* 1995) reported a significant increase in total N budget of mature seeds in response to added N fertilizer. They observed that the total N% in seeds derived from fertilizer was higher in higher dose of N. They further stated that NO_3 and NH_4 source of N was rapidly transported and readily incorporated into proteins. In present studies the decreased level of NH_4 source of N might be due to their rapid incorporation into protein and other nitrogenous organic compounds during the course of seed maturity. Results further indicated that by comparing the inoc with non-inoc treatment in particular doses of fertilizer, inoculation significantly but negatively influenced the NH_4-N of both year field seeds. Statistically significant and maximum amount of NH_4-N was also noted in T_2 inoc (0.9013 – 0.9780 g kg^{-1}) of both year field seeds, respectively. Whereas on the basis of marginal mean values the inoculation effect was noted as 18.91 and 12.57% lesser in both year field seeds when compared with their non-inoc treatments, respectively. Some other workers (Posypano and Zherukov, 1992; Ghobrial *et al.,* 1995) had also reported that inoculation and nodulation significantly increased the seed total N budget by 70 to 90% as compared with non-inoc soybean. Though our present findings were conflicting with the results obtained by aforesaid workers, but this decreased level of NH_4-N might be due to their rapid incorporation into protein and other nitrogenous organic compounds during the course of seed development to maturity. However, on the basis of grand mean values, the ES seed produced 27.89% greater NH_4-N than that of LS field seeds.

NO_2-N: Data recorded (Table 2) showed that fertilizer treatments in general significantly but adversely influenced the NO_2-N of LS seeds, while reverse was true in case of ES seeds when compared with treatment not receiving fertilizer (T_1). Statistically a maximum significant level is recorded in T_1 (3.950 g kg^{-1}) of LS and T_5 (1.968 g kg^{-1}) of ES field seeds. A very little is known about the NO_2-N status in the total N budget of mature soybean seeds in relation to added level of N fertilizer. However, most of the researchers reported that NO_2 absorption by soybean plant was 50% lesser than those of NO_3 or NH_4 (Singandhupe and Rajput, 1990; Ifsan, 1991; Pandey *et al.,* 1995). In present studies the higher level of NO_2-N than NO_3-N and NH_4-N in general and in LS in particular suggested that NO_2 was not preferentially and readily incorporated into various nitrogenous organic compounds during the entire course of development to maturity by seeds. Results also showed that by comparing the inoc with non-inoc in particular doses of fertilizer, inoculation significantly, positively affected the NO_2-N status and statistically a maximum significant level recorded in T_5 inoc (6.750 – 3.974 g kg^{-1}) of both year field seeds, respectively. However, on the basis of marginal mean values the inoculation effect was recorded as 198.80 and 67.00% greater in both year field seeds when compared with their respective non-inoc treatments. Many other workers also reported that N accumulation in all organs of soybean plant were 15.5 and 13.2% higher in inoc plants than those given N fertilizer only. Therefore, our results of both year field seeds are also in line with the findings recorded by Crozat *et al.* (1992), Posypano and Zherukov (1992) and Ghobrial *et al.* (1995). However, on the basis of grand mean values, the LS Field seed produced 29.55% increased NO_2-N than that of ES field seeds.

It is worth to mention that plant leaf is the primary organ in which all sources of mineral nitrogen (viz., NO_3-N, NH_4-N and NO_2-N) are metabolized and incorporated into various nitrogenous organic compounds. The metabolites are then transported and accumulated in seeds. Developing seed is the secondary organ of metabolism in which the imported inorganic mineral N are metabolized and incorporated into various nitrogenous organic molecules.

By summation the grand mean values of mineral N (i.e., NO_3-N, NH_4-N and NO_2-N), it can be safely concluded that 2nd year field seed produced lower level of mineral N than that of 1st year field seeds. This suggests that ES could enhance the incorporation of mineral N into protein and other nitrogenous organic compounds While by comparing the concentration of each source of mineral N, it was found that mature soybean seeds accumulated lesser NO_3-N followed by NH_4-N and NO_2-N. This suggests that during the course of N metabolism, leaf and developing seeds might have preferentially incorporated NO_3 first then followed by NH_4 and NO_2 source of nitrogen, respectively.

Correlation studies: The correlation coefficient (r) studies revealed that free amino acids of LS field seeds exhibited highly significant ($P<0.01$) positive correlation with soluble protein (0.824), oil content (0.618) and slightly significant ($P<0.05$) with NO_2-N (0.426), while ES field seed showed significant positive correlation with soluble sugars, NO_3-N (0.403) and NH_4-N (0.454), but negative (-0.599) with grain yield (Table 3). However, NO_3-N exhibited significant positive association with NO_2-N (0.592) and negative with starch content of (-0.724) LS seeds. But in case of ES field they showed significant positive association only with soluble sugars (0.595). Results also showed that NH_4-N exhibited non-significant association with all other remaining attributes of both year field seeds. However, NO_2-N exhibited significant positive correlation with oil content (0.382) and negative with starch content (-0.494) of LS seeds. But positive with starch content (0.454) of ES seeds, whereas none of the mineral N content exhibited either significant correlation with their respective grain yield. Research revealed that N concentration of soybean seed was positively correlated with grain yield. A significant positive association was also found between NO_3 content and seed yield. Therefore, our findings in term of correlation are not in accordance with the results obtained by Hanway and Weber (1971ab) and Liu and Hadley (1971), but to some extent are in conformity with the results of pot culture studies obtained by Achakzai and Kayani (2002b).

References

Achakzai, A.K.K. and S.A. Kayani, 2002a. Effect of fertilizer, inoculation and sowing time on the chemical composition of field grown soybean seeds. Asian J. Pl. Sci., 1: 618-621.

Achakzai, A.K.K. and S.A. Kayani, 2002b. Effect of fertilizer and inoculation on free amino acids and mineral nitrogen contents of pot culture mature soybean seeds. Pak. J. Agric. Res., (submitted).

Achakzai, A.K.K., S.A. Kayani, S. Jehan, M.A. Wahid and S.H. Shah, 2002. Effects of fertilizer, inoculation and sowing time on growth, yield and yield attributes of soybean under field conditions. Asian J. Pl. Sci., 1: 308-313.

Babich, A.A. and V.F.Petrichenko, 1992a. Factors increasing soybean productivity in the central Ukrainian forest steppe. Doklady-Vsesoyuznoi-Ordena-Lenina-I-Ordena-Trudovogo-Krasnogo-Znameni-Akademii-Sel's Kokhozyaistvennykh-Nauk-Imeni-V-1. Lenina, 5: 2-4.

Babich, A.A. and V.F. Petrichenko, 1992b. Photosynthetic activity and productivity of soybeans after liming, mineral fertilizer application and inoculation under forest steppe conditions in the Ukraine. Vestnik Sel's Kokhozyaistvennoi Nauki Moskva, 5-6: 110-117.

Bergerson, F.J., J.L. Turner, J.L. Peoples, M.B. Gault, R.R. Morthorpe and J. Brockwell, 1992. Nitrogen fixation during vegetative and reproductive growth of irrigated soybean in the field: Application of delta ^{15}N methods. Aust. J. Agric. Res., 43: 145-153.

Cataldo, D.A., M. Haroon, L.E. Schrader and V.L. Youngs, 1975. Rapid Colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Pl. Analysis, 6: 71-78.

Crozat, Y., A. Aveline and X. Pinochet, 1992. Proce. Sec. Cong. Europ. Soc. Agron. Warwick Univ. 23-28 Aug. 1992, Wellesbourne, UK, pp: 52-53.

Flamerz, S. and W.A. Bashir, 1981. Spectrophotometric determination of nitrite in waters. Analyst, February, pp: 243-247.

Ghobrial, W.M., D. Abadi, R.Y. Abdl-Fl-Kodoos and M.H. Hegazy, 1995. Nitrogen fixation potential as affected by *B. japonicum* strains and soybean cultivars for maximizing soybean productivity. Ann. Agric. Sci. Cairo., 40: 117-128.

Ham, G.E., I.E. Liener, S.D. Evans, R.D. Frazier and W.W. Nelson, 1975. Yield and composition of soybean seed as affected by N and S fertilization. Agric. J., 67: 293-297.

Hamilaton, P.B. and D.D. Van Slyke, 1943. Amino acids determination with ninhydrin. J. Biol. Chem., 150: 231-23.

Hanway, J.J. and C.R. Weber, 1971a. Dry matter accumulation in soybean (*Glycine max* (L) Merill) plants as influenced by N, P and K fertilization. Agro. J., 63: 263-266.

Hanway, J.J. and C.R. Weber, 1971b. N, P and K percentages in soybean [*Glycine max* (L.) Merill] plant parts. Agron. J., 63(March-April): 286-290.

Ifsan, D., 1991. Fertilizer nitrogen uptake by soybean as related to cultivars and time application using ^{15}N technique. J. Pl. Nutr., 14: 1369-1380.

Illahi, I., 1996. Plant Physiology: Bio-chemical Processes in Plants. University Grants Commission (UGC), H/9, Islamabad, Pakistan.

Lathwell, D.J. and C.E. Evans, 1951. Nitrogen uptake from solution by soybeans at successive stages of growth. Agron. J., 43: 264-270.

Liang, C.Q., Y.H. Zhang, Y.W. Zhang, L.G. Lu and L.Q. Zhu, 1991. Modernizing Agric., 2: 18-20.

Liu, M.C. and H.H. Hadley, 1971. Relationships of nitrate reductase activity to protein content in related nodulating and non-nodulating soybeans. Crop Sci., 11: 467-471.

Pandey, N.R., R. Dhurandher and R.S. Tripathi, 1995. Effect of irrigation and nitrogen on nodulation, N accumulation and seed yield of soybean (*Glycine max*). Ind. J. Agron., 40: 306-308.

Posypano, G.S. and B.Kh. Zherukov, 1992. Consumption of nutrient elements by soybean plants depending on symbiotic activity. Izvestiva Timiryazevskopi-Sel' Skokhozyaistvennoi Akademii., 4: 196-202.

Qiu, Z.C., G.P. Liu and Y.Q. Zhu, 1987. Determination of water-soluble ammonium ion in soil by spectrometry. Analyst, 112: 909-911.

Salisbury, F.B. and C.W. Ross, 1992. Plant Physiology. Wadsworth Publ. Co., Belmont, California, USA.

Singandhupe, R.B. and R.K. Rajput, 1990. Nitrogen use efficiency in rice under varying moisture regimes, sources and levels in Semi-reclaimed Sodic Soil. Ind. J. Agron., 35: 73-81.

Steel, R.G.D. and V.H.J. Torrie, 1980. Principles and Procedures of Statistics. McGraw-Hill publishers. U.K., pp: 481.

Sugimoto, T., K. Sueyoshi and Y. Oji, 1997. Increase of PEPC activity in developing rice seeds with nitrogen application at flowering stage. T. Ando ***et al.*** (Eds.), Plant nutrition for sustainable food prod. environ., pp: 811-812.

Sugimoto, T., K. Nomura, R. Masuda, K. Sueyoshi and Y. Oji, 1998. Effect of nitrogen application at the flowering stage on the quality of soybean seeds. J. Pl. Nutri., 21: 2065-2075.

Ting, I.P., 1982. Plant Physiology. Addison Wesley Services in Life Sciences. New York, USA.