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Effect of Fertilizer and Inoculation on Mineral Nitrogen Contents of Pot Culture Mature Soybean Seeds

Abdul Kabir Khan Achakzai and Safdar Ali Kayani
Department of Botany, University of Balochistan, Quetta, Pakistan

Abstract: One year Pot culture experiment on Soybean in Soil + FYM (3:1) was conducted in medium textured and salt free soil of Quetta, Balochistan. Seven different treatments (T) of fertilizer were applied to both non-inoculated (non-inoc) and inoculated (inoc) set of experiment. T_1 was kept control; T_2 contained 23 + 60 + 30 kg NPK ha^{-1} and from T_3 to T_7 N fertilizer in the form of urea was added @ 25, 50, 75, 100 and 125 kg ha^{-1} along with combination of the same constant dose of P_2O_5 and K_2O respectively. After physiological maturity, seeds were analyzed for their mineral N contents. Results showed that fertilizer treatments in general significantly, but negatively influenced the NO_3-N and NH_4-N, but reverse was true for NO_2-N. A minimum level of NO_3-N was recorded in T_4 (0.065 μM), NH_4-N in T_7 (0.133 g kg^{-1}) and a maximum level of NO_2-N in T_5 (1.864 g kg^{-1}). While by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general significantly and positively influenced the total mineral N content of mature soybean seeds. A maximum level of NO_3-N was noted in T_4 (0.122 μM), NH_4-N in T_1 (0.466 g kg^{-1}) and NO_2-N in T_4 (4.314 g kg^{-1}). However, by comparing the marginal mean values, inoc seeds comparatively produced greater amount of total mineral N content than that of non-inoc seeds. It is therefore safely concluded that low level of NO_3-N followed by NH_4-N in non-inoc seeds might have preferentially incorporated it into various nitrogenous organic compounds during the entire course of seed development to physiological maturity. The correlation coefficient (r) studies revealed that NO_3-N and NO_2-N were significantly and positively correlated with oil (0.445 and 0.489), but non-significantly correlated with protein (-0.020 and 0.041) and grain yield (0.210 and -0.206) respectively. While reverse was true in case of NH_4-N association.

Key words: Soybean, fertilizer, inoculation, NO_3-N, NH_4-N, NO_2-N, correlation

Introduction

Soybean (***Glycine max*** L.) is mainly produced for protein and oil contents. It contains 40-42% good quality proteins and 18-22% oil comprising up of 85% unsaturated fatty acids and is free from cholesterol. Soybean not only contains high quality protein, but their protein content is also much higher than that of other foods. So it is highly desirable in human diet and animal nutrition (Haq ***et al.***, 2002).

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_4N and NO_2-N) status of mature soybean seeds in response to various level of added fertilizer (with and without inoculation). 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 (Achakzai ***et al.***, 2003a).

Research revealed that N concentration of soybean and rice grain increased with increase in N dose respectively (Lathwell and Evans, 1951; Singandhupe and Rajput, 1990). They 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 (1971a, 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 was15.5% 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 aimed to evaluate the beneficial effect of added N fertilizer (with and without inoculation) on the mineral N status 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

One year Pot culture experiment on soybean cv.Williams-82 was carried out in Soil + FYM (3:1) in Botanical Garden, University of Balochistan, Quetta. The soil used was of medium textured and salt free. The FYM was also analyzed for their NPK composition, which was found as 0.75%, 0.18% and 0.25% respectively. The following seven different treatments (T) of fertilizer were applied to both non-inoculated (non-inoc) and inoculated (inoc) set of experiments.

T_1 = No seed inoculation + No fertilizer (control)
T_2 = No seed inoculation + 23-60-30 kg NPK ha^{-1}
T_3 = No seed inoculation + 25-60-30 kg NPK ha^{-1}
T_4 = No seed inoculation + 50-60-30 kg NPK ha^{-1}
T_5 = No seed inoculation + 75-60-30 kg NPK ha^{-1}
T_6 = No seed inoculation + 100-60-30 kg NPK ha^{-1}
T_7 = No seed inoculation + 125-60-30 kg NPK ha^{-1}

T_1 = Seed inoculation + No fertilizer (control)
T_2 = Seed inoculation + 23-60-30 kg NPK ha^{-1}
T_3 = Seed inoculation + 25-60-30 kg NPK ha^{-1}
T_4 = Seed inoculation + 50-60-30 kg NPK ha^{-1}
T_5 = Seed inoculation + 75-60-30 kg NPK ha^{-1}
T_6 = Seed inoculation + 100-60-30 kg NPK ha^{-1}
T_7 = Seed inoculation + 125-60-30 kg NPK ha^{-1}

The source, time and methods of fertilizer application have already explained by Achakzai and Kayani (2002). The seeds of each treatment were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown coloration. Finally the seeds of each treatment were ground in a grinder, sieved through Mesh No. 60 (Johnson and Firth Brown Ltd. London).

Buffer extraction

Air-dried defatted soybean seed powder (0.2g) from each sample was homogenized in 20.0 ml of phosphate buffer solution (0.1M, pH 7.0) at room temperature for 16 hrs, 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 4.0^{0}C and then used for the determination of the following chemical components.

Nitrate nitrogen (μM)

Nitrate was determined spectrophotometrically 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 in conc. sulfuric acid) was added in a test tube. After 20 min 4.3 ml of NaOH (2.0 M) was also added in it and the contents of the tube were thoroughly mixed. The absorbance was monitored at 410 nm using Spectrophotometer (Hitachi U-1100, Japan). A series of 5 different standard solutions (0.125 to 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 nitrogen (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) 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 hour 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 to 0.5 mM) from the stock solution of NH_4Cl (0.01M) were prepared. A calibration graph of the standard solutions was drawn and used for calculation of NH_4-N in the sample.

Nitrite nitrogen (g kg^{-1})

Nitrite was also spectrophotometrically determined following the procedure adopted 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% w/v) and 0.5 ml naphtha-1-ol solution (0.35% w/v). 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 (5.0 to 25.0 ppm) from the stock solution of sodium nitrite (1000 ppm) were prepared. A

calibration graph of the standard solutions was also drawn and used for the calculation of NO_2-N in the sample.

The data obtained were statistically calculated following the procedure described by Steel and Torrie (1980). MSTAT-C Computer software package for statistical analyses was used for calculation of analysis of variance (ANOVA) and Least Significant Difference (LSD) test. Simple correlation coefficient (r) studies were also worked out for all mentioned nutrients as well as with other chemical components and grain yield in mature soybean seeds, which has been already explained Achakzai and Kayani (2002) and Achakzai ***et al.*** (2003b).

Results and Discussion

Data presented in Table 1 showed that in response to different level of fertilizer with and without inoculation and its interactions exhibited highly significant ($P<0.01$) effect on total mineral N contents of mature soybean seeds, whereas replications exhibited non-significant effect.

NO_3-N

Data regarding mean values (Table 2) showed that fertilizer treatments in general significantly but adversely affected the NO_3-N (except T_5 and T_6) when compared with treatment not receiving fertilizer (T_1). Result also showed that by comparing the inoc with non-inoc treatment in particular doses of fertilizer, inoculation in general significantly and positively influenced the NO_3-N of mature soybean seeds and statistically a maximum significant level is recorded in T_4 treatment (0.122 μM). While, on the basis of marginal mean values, the inoculation effect is noted as 25.30% greater over non-inoc treatment. Besides protein, oil and sugars, the elemental composition of food grains is also important in human and animal nutrition. Though a good deal of work has been carried out on the effects of applied fertilizer and inoculation 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 applied 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). It is possible that in developing seeds the NO_3 source of N in response to fertilizer was increasingly incorporated into proteins and other nitrogenous organic compounds on the maturity of seeds. Most of the researchers have also reported an increase in total seed N yield of inoc when compared 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) and Ghobrial ***et al.*** (1995).

NH_4-N

Data pertaining to mean values (Table 2) showed that N fertilizer in general significantly but adversely affected the NH_4-N content of seeds. Statistically a significant minimum amount is noted in T_7 (0.133 g kg^{-1}). 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

Table 1: Analysis of variance (ANOVA) for mineral N content of Pot culture mature soybean seeds in response to fertilizer treatments alone (A) and in combination with inoculum (B)

	F-value of variables at an error of 26				
Variable	Replications df = 2	Fertilizer (A) df = 6	Inoculum (B) df = 1	A X B df = 6	CV (%)
NO_3-N (μM)	0.8822ns	38.017*	343.228*	55.460*	3.93
NH_4-N (g kg^{-1})	0.6925ns	726.777*	792.702*	92.679*	3.49
NO_2-N (g kg^{-1})	1.3582ns	9115.000*	159064.00*	5854.000*	0.90

* significant at 1% level of probability, ns = non-significant. CV = Coefficient of variation.

Table 2: Effect of various level of fertilizer on the mineral N composition of Pot culture non-inoculated and inoculated mature soybean seeds

Treatments	NO_3-N (μM)	NH_4-N (g kg^{-1})	NO_2-N (g kg^{-1})
T_1 (non-inoc)	0.07667e	0.3450b	0.3047m
(Inoc)	0.07767e	0.4663a	1.8170g
T_2 (non-inoc)	0.06767g	0.1707ef	0.4490k
(Inoc)	0.07430ef	0.3377b	2.5650c
T_3 (non-inoc)	0.06233h	0.3190c	0.5953j
(Inoc)	0.09133b	0.3450b	3.5750b
T_4 (non-inoc)	0.06467gh	0.1590f	0.6360i
(Inoc)	0.12200a	0.3053c	4.3140a
T_5 (non-inoc)	0.07767e	0.1763de	1.8640f
(Inoc)	0.09467b	0.1877d	3.5840b
T_6 (non-inoc)	0.08500c	0.1557f	1.3520h
(Inoc)	0.09233b	0.1773de	1.9650d
T_7 (non-inoc)	0.07167f	0.1330g	0.4180l
(Inoc)	0.08133d	0.1607f	1.9120e
LSD (5%)	0.003357	0.01501	0.02907
LSD (1%)	0.004538	0.02029	0.03930
MM (non-inoc)	0.07224	0.2084	0.8027
MM (inoc)	0.09052	0.2829	2.8189
Grand Mean	0.081	0.246	1.811

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.

fertilizer. They observed that the percentage of 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 rapid incorporation of NH_4 into protein and other nitrogenous organic compounds during the course of seed maturity. While by comparing the inoc with non-inoc treatment in particular doses of fertilizer, inoculation significantly increased the NH_4-N level of seeds and a maximum significant amount is recorded in T_1 (0.466 g kg^{-1}). Whereas on the basis of marginal mean values the inoculation effect was recorded as 35.75% greater over non-inoc treatments. Some other workers (Posypano and Zherukov, 1992; Ghobrial ***et al.***, 1995) have also reported that inoculation and nodulation significantly increased the seed total N by 70 to 90% as compared with non-inoc soybean. Though apparent nodules were not established in any set

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

Variables	1	2	3	4	5	6	7	8
1	1.000							
2	0.102ns	1.000						
3	0.825**	0.254ns	1.000					
4	-0.020ns	-0.489**	0.041ns	1.000				
5	0.445*	-0.423ns	0.489**	0.716**	1.000			
6	0.033ns	-0.760**	-0.117ns	0.137ns	0.325ns	1.000		
7	-0.602**	0.074ns	-0.642**	-0.353ns	-0.591**	-0.148ns	1.000	
8	0.210ns	-0.528**	-0.206ns	0.207ns	0.468*	0.610**	-0.281ns	1.000

* and ** significant at $P<0.05$ and $P<0.01$ respectively, while ns stands for non-significant at both level of probability. (1) NO_3-N, μM. (2) NH_4-N, g kg^{-1}. (3) NO_3-N, g kg^{-1}. (4) Soluble protein, g kg^{-1}. (5) Oil contents, g kg^{-1}. (6) Soluble sugars, g kg^{-1}. (7) Starch contents, g kg^{-1}. (8) Yield Pot^{-1}, g.

of the present experiment (as already explained by Achakzai and Kayani, 2002), but still our findings regarding inoculation are in conformity with the results obtained by earlier workers.

NO_2-N

Results pertaining to mean values (Table 2) showed that fertilizer treatments significantly and consistently increased the NO_2-N and statistically a maximum significant amount was recorded in T_5 dose of N fertilizer (1.864 g kg^{-1}). A very little is known about the NO_2-N status in the total N budget of mature soybean seeds in relation to added N fertilizer. However most of the researchers reported that NO_2 absorption by soybean plant was 50% lesser than those of NH_4 or NO_3 (Ifsan 1991; Pandey ***et al.***, 1995). In present studies the higher level of NO_2-N than NO_3-N and NH_4-N suggests that NO_2 was not preferentially and readily incorporated into nitrogenous organic compounds during the entire course of development to maturity by seeds. By comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly and positively influenced the NO_2-N and statistically a maximum significant level was recorded in T_4 inoc dose (4.314 g kg^{-1}). Whereas on the basis of marginal mean values, the inoculation effect was recorded as nearly 2.5 fold greater than non-inoc treatments. Many other workers have also reported that N accumulation in all organs of soybean plant was 15.5% and 13.2% higher in inoc plants than those given N fertilizers only. Therefore our results are also in line with the findings recorded by many researchers (Crozat ***et al.***, 1992; Posypano and Zherukov, 1992; Ghobrial ***et al.***, 1995; Achakzai ***et al.***, 2003a).

It is worth to mention that plant leaf is the primary organ in which all sources of mineral nitrogen (i.e., NO_3, NH_4 and NO_2) 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.

Based on grand mean values, it can be safely concluded that the low level of NO_3-N suggests that soybean plants might have preferentially and readily incorporated it into various nitrogenous organic compounds as compared it with NH_4-N and NO_2-N respectively.

Correlation

The correlation coefficient (r) studies revealed that NO_3-N exhibited significant positive association with oil (0.445) and NO_2-N contents (0.825), but negative with starch contents (-0.602). However, NH_4-N showed highly significant negative association with protein (-0.489), soluble sugars (-0.760) and grain yield (-0.528) (Table 3). Whereas NO_2-N also exhibited significant positive correlation with oil (0.489) and negative with starch contents (-0.642). Research revealed that N concentration of soybean seed was positively correlated with grain yield. A significant positive correlation was also observed between NO_3 content and seed yield. Therefore, our findings are not in agreement with the results obtained by Hanway and Weber (1971a, b) and Liu and Hadley (1971), but are in line with the results obtained by Achakzai ***et al.*** (2003a).

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