Journal of Biological Sciences 3 (10): 882-891, 2003
ISSN 1727-3048

Chemical Composition of Soybean cv. Williams-82 Seeds in Pot Culture Experiments

Abdul Kabir Khan Achakzai, Safdar Ali Kayani, [1]Muhammad Yaqoob and [1]Abdul Nabi
Department of Botany, [1]Department of Chemistry,
University of Balochistan, Quetta, Pakistan

Abstract: A pot culture experiment on soybean cv. Williams-82 was conducted in clay loam soil with six different level (T_2 to T_7) of added N fertilizer @ 23, 25, 50, 75, 100 and 125 kg ha^{-1} plus a constant dose of P_2O_5 + K_2O fertilizer @ 60 + 30 kg ha^{-1}, respectively Whereas T_1 with zero level of added NPK was kept control. A constant dose of FYM was also mixed with soil (3:1). These six fertilizer treatments were applied to both non-inoculated and inoculated pot culture crops. Results showed that fertilizer treatments significantly increased the protein and oil content of seeds. A maximum oil content (18.42%) was recorded in T_6 dose of fertilizer. While soluble sugars are significantly increased only in higher doses of fertilizer (T_4 to T_7), Whereas the starch content and free amino acids showed significant decrease. As far inoculation effects are concerned, they generally did not significantly influenced the protein content and soluble sugars, but oil content (19.09%) and free amino acids respond positively. While reverse was true for starch contents. The correlation coefficient (r) studies revealed that protein content exhibited highly significant positive association with oil (0.716) without any concomitant loss in grain yield. Which could be used a suitable selection criterion for predicting the quality and quantity of grain yield in soybean cv. Williams-82.

Key words: Soybean, fertilizer, inoculation, oil, protein, soluble sugars, starch, amino acids, correlation

Introduction

Soybean ***(Glycine max*** L.***)*** is one of the most important oil and protein crops in the world. In Pakistan edible oil is the largest bill next to motor oil or it is the single largest food import item and the import bill is continuously increasing due to a constant increase in annual per capita consumption and the rising international price trend (Bhutta ***et al.***, 1995). In Pakistan it is non-conventional oil seed crop (Shah ***et al.***, 1999). It contains 40-42% good quality protein and 18-22% oil comprising up of 85% unsaturated fatty acid 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 (Anonymous, 1994) and it also contains 12% carbohydrate (Anonymous, 1996).

Nitrogen is a fourth important major plant constituent of many important macromolecules. The soils of Pakistan in general and of Balochistan in particular are deficient in available N. They needs a sufficient amount of N fertilizer for non-leguminous and a small amount (as a starter dose) for leguminous crops (Anonymous, 1995). Leguminous plants in association with ***Rhizobium*** species have the potential to fix large amounts of N_2 which contributes to the soil N pool provided that the N_2 fixation is not restricted by any other environmental or microbial factors (Jefing ***et al.***, 1992; Ali and Hussain, 1996). Though soils of Pakistan have either nil or very low viable count of effective rhizobia and low N-supplying capacity because of low organic matter levels (0.3-1.0%). Yet only 1.0% of the total legume crops are inoculated (Ladha ***et al.***, 1996; Aslam ***et al.***, 1997 and 2000).

Several researchers recorded different responses of soybean seed protein, oil and other components in response to various level of added of NPK fertilizers (Babich and Petrichenko, 1992ab; Khushwaha and Chandel, 1997; Sugimoto ***et al.***, 1998). Breeding trials frequently revealed that, seed protein tends to be inversely or negatively correlated to both seed yield and oil concentration (Quattava and Weaver, 1994; Liu ***et al.***, 1995; Wilcox and Zhang, 1997; Wilcox, 1998). While, Tinius ***et al.*** (1993) explained that correlation between seed yield and its composition are often found significant.

The present study was therefore mainly designed to determine the optimum level of nitrogen fertilizer used (with and without inoculum) and its effects on oil, protein, soluble sugars, starch and free amino acid contents of mature soybean seeds. The data was also statistically analyzed to furnish the information on the nature of association among different chemical components to choose a suitable selection criteria for predicting the quality and quantity of grain yield in soybean cv. Williams-82.

Materials and Methods

Pot culture experiments on soybean [***Glycine max*** (L.) Merill] cv. Williams-82 were carried out during the 1st week of July 1996 in Botanical Garden, University of Balochistan, Quetta, Pakistan. Thoroughly cleaned earthen 42 pots (452.5 cm^2 each) were used in the experiment. The pots were labeled according to their respective treatments and replicated thrice. A composite soil sample (0.0 to 30 cm depth) was mixed with decomposed farmyard manure (FYM) with 3:1 ratio respectively. The NPK composition of FYM was determined as 0.75, 0.18 and 0.25% respectively. An equal volume of such soil was added in each pot and irrigated with an equal volume of water. All pots were grouped into two sets, one for inoculated (inoc) and other for non-inoculated (non-inoc) experiments. In each inoculated pot twelve healthy and uniform seeds of soybean cv. Williams-82 were sown at an equal distance and at a depth of 3.0 cm. The same was also repeated for non-inoculated pots. For inoculated set, seeds were treated (just before sowing in the shade) with fresh inoculum of ***Rhizobium japonicum*** strain SL2 @ 8.0 g kg^{-1} seed following the procedure adopted by Anonymous (1984). All pots were mulched with a thin layer of washed wheat straw. After the completion of germination, plants were thinned to eight and plant-to-plant distance was maintained at 7.5 cm apart in each pot. All pots were brought out in an open field by discarding their wheat straw, equally irrigated (when needed) and arranged them in a randomized complete block design (RCBD). The treatments used were as follows.

T_1 = No seed inoculation + No fertilizer (control) T_1 = 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_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 urea, diammonium phosphate (DAP) and sulphate of potash (SOP) were used as a source of N, P and K fertilizers, respectively. A full dose of DAP, SOP and a half dose of urea was dissolved separately in water by irrigating the respective pots just after thinning. Whereas, the remaining half dose of urea was also dissolved in water by irrigating the respective pots but two weeks after thinning. Finally the mature dry seeds of all treated plants were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown coloration.

10.0 g air-dried seed of each sample was then ground separately in a grinder and sieved through mesh No. 60 (Johnson and Firth Brown Ltd. London).

Before analyses, 0.2 g air-dried seed powder of each sample was homogenized in 20 ml of phosphate buffer solution (0.1 M, pH 7.0) at room temperature for 16 hrs, with continuous shaking at 300 rpm (Edmond Bühler 7400 Tübingen). Each sample was centrifuged at 5,000 rpm using IEC B-20A Centrifuge (Damon/IEC Division) for 20 min, filtered through Whatmann filter paper, stored at 4^0C and then used for the determination of the following chemical components.

Oil determination (g kg^{-1})

Oil contents were determined as described by Illahi and Jabeen (1992). Air-dried (3.0 g) Soybean powder was homogenized in 27 ml diethyl ether at room temperature for 24 hrs with continuous shaking at the rate of 250 rpm, filtered through Whatmann filter paper. The solvent was evaporated at 70 ^{0}C using water bath. Oil content was then calculated as under.

$$\text{Oil contents (g kg}^{-1}\text{)} = \frac{\text{Weight of oil}}{\text{Weight of sample taken}} \times 1000$$

Protein determination (g kg^{-1})

The soluble protein of defatted powder was determined by using Biuret method (Harris and Angal, 1989) and bovine serum albumin (BSA) was used as a stock solution of protein and the absorbance was monitored at 540 nm using a Spectrophotometer (Hitachi U-1100, Japan).

Soluble sugars and starch determination (g kg^{-1})

They were determined following Malik and Srivastava (1979) method. For the estimation of soluble sugars, air-dried defatted powder (0.3 g) was suspended in 25 ml ethanol (80%) with continuous shaking for 1 hr and filtered through Whatmann filter paper. The residue was resuspended in ethanol to remove all the traces of soluble sugars and filtered. The filtrate was diluted to 100 ml with de-ionized water and used for the determination of soluble sugars. The residue of the filtrate left on the filter paper was used for the determination of starch by mixing in 5.0 ml of water and 6.5 ml perchloric acid (52%) with continuous shaking for 1 hr and filtered. This process was repeated twice and the volume of the filtrate was adjusted to 100 ml with water and used for the determination of starch. Each filtrate sample (0.2 ml) was taken in a test tube and treated it carefully with 4.0 ml of anthrone reagent (0.2 % w/v) and placed on boiling water bath for 20 min. After cooling, the absorbance of each sample was monitored at 625 nm using the same Spectrophotometer. Total soluble sugars or starch were then calculated as under.

$$\text{Soluble sugars or starch } (\text{g kg}^{-1}) \text{ air dry weight} = \frac{\text{Conc. of glucose solution} \times \text{Abs. of sample} \times \text{Dilution factor}}{\text{Absorbance of glucose}}$$

Free amino acids determination (g kg^{-1})

The total free amino acids of defatted powder were determined spectrophotometrically following the procedure of Hamilaton and Van Slyke (1943). Sample (1.0 ml, buffer extract) was placed in a test tube, mixed in a mixture of 1.0 ml ninhydrin and 1.0 ml of pyridine solution. 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. Total free amino acids were then calculated as under.

$$\text{Total free amino acids } (\text{g kg}^{-1}) \text{ air dried defatted seeds} = \frac{\text{Abs. of sample} \times \text{Vol. of sample} \times \text{Dilution factor}}{\text{Weight of the sample}} \times 1000$$

The data obtained were statistically analyzed 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) Tables and least significance difference (LSD) test using the experimental model number 8 under factorial randomized complete block design (RCBD). Simple correlation coefficient (r) statistical test was also applied for all above-mentioned parameters following the Fisher and Yates (1953) statistical Table of significance.

Results and Discussion

Data presented (Table 1) showed that in response to different level of fertilizer alone and in combination with inoculum exhibited highly significant effect ($P<0.01$) on all mentioned chemical components of soybean seeds. While interaction between fertilizer and inoculum was also found significant (except soluble sugars).

Table 1: Analysis of variance (ANOVA) for chemical composition of pot culture Soybean [*Glycine max (L.)* Merill] cv. Williams-82 seeds in response to added fertilizer alone (A) and in combination with inoculum (B)

	F-Value of variables at an error of 26					
Variables (g kg^{-1})	Replications (Df = 2)	Fertilizer (A) (Df = 6)	Inoculum (B) (Df = 1)	A x B (Df = 6)	CV (%)	Grand/ Mean
1) Oil content	0.5652ns	47.7135**	83.5341**	2.4766*	1.44	176.721
2) Protein	0.8947ns	237.690**	17.995**	1.534**	0.92	327.074
3) Soluble sugars	0.8814ns	31.2982**	13.7704**	2.1487ns	11.01	28.526
4) Starch content	1.8625ns	225.487**	722.277**	29.020**	1.06	139.268
5) Free amino acids	0.2549ns	718.05**	3365.29**	106.79**	2.19	0.037

* and ** significant at P< 0.05 and P< 0.01 respectively, while ns = non-significant and Df = degree of freedom.

Table 2: Effect of various levels of fertilizer without inoculum (non-inoc) and with inoculum (inoc) on various chemical component of pot culture Soybean [*Glycine max* (L.) Merill] cv. Williams-82 seeds

	Chemical Components (g kg^{-1})				
Treatments	Oil contents	Protein	Soluble sugars	Starch	Free amino acids
T_1 (non-inoc)	161.0i	301.8g	21.12bc	150.5b	0.037d
(inoc)	170.9g	306.8f	21.17bc	133.1gh	0.056a
T_2 (non-inoc)	180.3cd	356.4a	22.31bc	141.6d	0.038cd
(inoc)	181.3bcd	359.2a	17.45c	131.8h	0.059a
T_3 (non-inoc)	166.1h	307.5f	23.32b	167.4a	0.031f
(inoc)	175.7ef	307.3f	21.19bc	144.5c	0.039cd
T_4 (non-inoc)	170.1gh	315.8e	36.39a	134.6fg	0.033ef
(inoc)	179.9cde	320.6e	25.30b	123.2j	0.052b
T_5 (non-inoc)	177.2def	327.8d	36.92a	135.4efg	0.021i
(inoc)	185.5b	332.8cd	32.81a	127.7i	0.041c
T_6 (non-inoc)	184.2bc	335.9c	37.69a	137.7e	0.025gh
(inoc)	190.9a	344.8b	34.97a	134.3fg	0.036de
T_7 (non-inoc)	173.0fg	330.5d	34.52a	152.1b	0.023hi
(inoc)	178.1de	331.6cd	34.19a	136.2ef	0.027g
LSD (5%)	4.273	5.024	5.273	2.485	0.003357
MM (non-inoc)	173.13	325.10	30.32	145.61	0.030
MM (inoc)	180.33	329.01	26.73	132.93	0.044

Mean values in a column followed by the same letter(s) do not differ significantly at 5% level of probability.
MM = marginal mean values. Non-inoc = non-inoculated. Inoc = inoculated.

Table 3: Correlation coefficient (r) studies of various chemical components of pot culture soybean cv. Williams-82 seeds in response to different level of added nitrogen (with and without inoculation) fertilizer

Components (g kg^{-1})	1	2	3	4	5	6
1) Protein	1.000					
2) Oil contents	0.716 **	1.000				
3) Soluble sugars	0.325 ns	0.137 ns	1.000			
4) Starch -0.591 **	-0.353 ns	-0.148 ns	1.000			
5) Free amino acids	0.070 ns	0.067 ns	-0.689 **	-0.429 *	1.000	
6) Grain yield (g pot^{-1})	0.468 *	0.207 ns	0.610 **	-0.281 ns	-0.374 *	1.000

* and ** significant at P (0.05) and P (0.01), respectively. While, ns stand for non-significant.

Oil content

Data presented (Table 2) showed that fertilizer treatment significantly and positively influenced the oil content of soybean seeds. A maximum oil content was noted in T_6 (184.2 g kg^{-1}) followed by T_2. This suggests that added fertilizer significantly increased the oil content and is strongly in conformity with the results obtained by Jana ***et al.*** (1990) and Sugimoto ***et al.*** (1998). Results also showed that by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation significantly increased the oil content (except T_2). Even though nodules were apparently found to be absent in any set of experiment (Achakzai and Kayani, 2002a), but inoculation still showed their silent positive effect which are in support of the findings of Pourdavai and Yousefi (1993).

Soluble protein

Data pertaining to mean separation values (Table 2) showed that fertilizer treatment significantly and positively affected the protein content of seeds. A maximum protein content was recorded in T_2 (356.4 g kg^{-1})) followed by T_6. These findings are in accordance with the results obtained by Jayapaul and Ganesaraja (1990). On the other hand few workers reported that though protein content of seeds were increased by added N fertilizer, but this increase was statistically non-significant (Khushwaha and Chandel, 1997; Sugimoto ***et al.***, 1998). Result also revealed that by comparing the inoculated with non-inoculated treatment in particular doses of fertilizer, inoculation generally did not significantly affect the seed protein contents. This might be due to deceived nodulation (Achakzai and Kayani, 2002a) and are in contradiction with the findings of Pourdavai and Yousefi (1993) and Gretzmacher ***et al.*** (1994).

Soluble sugars

Data regarding mean separation values (Table 2) exhibited that higher doses of fertilizer (T_4 to T_7) significantly and positively affected the soluble sugars. While lower doses did not significantly affect when compared with their check (T_1). Though no extensive studies have been carried out on the effects of added N fertilizer on soluble sugar of mature soybean seeds. However, few researchers reported that even though mineral composition of soybean is fairly good, but it is notorious for its low carbohydrate content, having an average of 9.95% soluble sugars (Khan, 1990; Hartwig ***et al.***, 1997). In present studies the marginal mean values of non-inoculated treatments are 3.03% that are far lesser than those reported by aforesaid workers. Therefore, this low level of soluble sugars could increase the quality of soybean seeds. Results also showed that by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation in general did non-significantly affect the soluble sugars. The marginal mean value of soluble sugars in inoculated treatments were calculated as 2.67%, which are also far lesser than those reported by aforesaid workers. Research revealed that oligosaccharides (viz., raffinose, stachyose and verbascose) are frequently found in soybean and are the causative factors for the flatulence character of soybean meal. These saccharides are water soluble and can not be digested by human small intestine as the galactosidase enzyme, which breaks down these carbohydrates are lacking in human digestive system (Khan, 1990).

Therefore, in present studies the decreased level of total soluble sugars either in non-inoculated or in inoculated set of experiment could increase the quality and decrease the flatulence character of soybeans.

Starch

Data presented (Table 2) showed that fertilizer treatment in general and inoculation in particular significantly but adversely affected the starch content of seeds. Data further revealed that on the basis of marginal mean values, non-inoculated seed produced 14.56% and inoculated seed produced 13.29% starch. While Khan (1990) reported that soybean is notorious for its low carbohydrate contents and having an average of 4.32% starch. Whereas in present studies the average value of starch is far greater than that reported by Khan (1990), which could ultimately provide a better source of energy for both human diet and poultry farms without creating any digestive disturbances. Therefore, it can be safely concluded that the decreased level of soluble sugars and increased level of starch could increase the value and quality of soybean meal.

Free amino acids

Data pertaining to mean separation values (Table 2) exhibited that fertilizer treatments in general significantly but negatively influenced the free amino acid contents when compared with their control (T_1). Whereas by comparing the inoculated with non-inoculated treatments in particular doses of fertilizer, inoculation significantly increased the free amino acid contents. However, on the basis of marginal mean values, the inoculation effects were noted as 46.67% greater over non-inoculated treatments. Though no extensive work has been done on the effects of various doses of added N fertilizer on the free amino acid level of mature soybean seeds. However, few researchers stated that application of various level of added N fertilizer resulted a drastic reduction in the contents of total and some amino acids in developing seeds and also led to faster accumulation of oil content in mature soybean seeds. Therefore, our findings are in confirmation with the results obtained by Sugimoto ***et al.*** (1998), but in contradiction with Babich and Petrichenko (1992 ab) and Sugimoto ***et al.*** (1997).

Correlation

The simple correlation coefficient (r) studies revealed that protein content exhibited significant positive association with oil (0.716) and insignificant with yield and other chemical attributes. While oil content and soluble sugars showed significant positive association with grain yield (Table 3). Although seed yield and seed protein are both heritable traits. However, breeding trials frequently revealed a negative or non-significant correlation between these two traits. Increasing seed protein concentration without a concomitant loss in either seed oil or seed yield should increase the value of soybean. Research studies also revealed that seed protein tends to be either inversely or insignificantly related to both seed yield and oil concentration, while seed oil tends to be inversely related with protein but directly related with yield. Therefore, our findings are to great extent in confirmation with the findings recorded by Tinius ***et al.*** (1993) and to some extent with many others (Quattava and Weaver, 1994; Liu ***et al.***, 1995; Hartwig ***et al.***, 1997; Wilcox and Zhang, 1997; Wilcox, 1998). Data further revealed that free amino acids exhibited significant but inverse relationship with soluble sugars, starch and yield. These findings are in contradiction with the reports explained by Sugimoto ***et al.*** (1998). Thus it can be safely concluded that in response to added fertilizer (with and without inoculum), protein content exhibited significant positive association with oil without any concomitant loss in their grain yield.

References

Achakzai, A.K.K. and S.A. Kayani, 2002a. Effect of fertilizer and inoculation on nodulation, growth, yield, yield attributes and correlation studies of pot culture soybean cv. Williams-82. Pak. J. Agric. Res., (accepted).

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

Ali, A. and A. Hussain, 1996. Effect of nitrogen application on the growth of ***Sesbania bispinosa***. Pak. J. Bot., 28: 85-89.

Anonymous, 1984. Legume Inoculants and Their Use. Food and Agriculture Organization (FAO) of the United Nations. Rome, Italy.

Anonymous, 1994. Vegetable Soybean. Grow meat in your fields. Oil seeds Programme, NARC, Pakistan Agricultural Research Council (PARC), Islamabad, Pakistan.

Anonymous, 1995. Proceedings of the training on balanced fertilizer use and the environment, (17-20 Oct. 1993), Govt. of Pakistan P and D. Division, National Fertilizer Development Centre (NFDC), Islamabad, Pakistan, pp: 25-37.

Anonymous, 1996. Soybean Yield Gap Analysis In Pakistan. National Agricultural Research Centre (NARC), Islamabad, Pakistan.

Aslam, M., I.A. Mahmood, S. Ahmad, M.B. Peoples and D.F. Herridge, 1997. Surveys of chickpea N_2 fixation in the Potohar and Thal areas of the Punjab, Pakistan. In: Extending nitrogen fixation research to farmer's fields. International workshop on managing legume nitrogen fixation in the cropping systems of Asia, 20-24. ICRISAT, Asia Centre, India, pp: 353-360.

Aslam, M., I.A. Mahmood, T. Sultan and S. Ahmad, 2000. Inoculation approach to legume crops and their production assessment in Pakistan-A Review. Pak. J. Biol. Sci., 3: 193-195.

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.

Bhutta, A.R., S.I. Ahmad and M.H.R. Bhatti, 1995. Edible oilseed industry development in Pakistan. Sci. Tech. and Dev., 14: 20-26.

Fisher, R.A. and F. Yates, 1953. Statistical Tables for Biological, Agricultural and Medical Research. 4th Ed. Oliver and Boyd, Edinburgh.

Gretzmacher, R., N. Schahbazian and H. Pourdavai, 1994. Influence of symbiotic organic and mineral nitrogen on yield and quality of soybeans. Bodenkultur, 45: 253-267.

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

Harris, E.L.V. and S. Angal, 1989. Protein Purification - A Practical Approach. 1st Ed., IRL Press, Oxford, UK.

Hartwig, E.E., T.M. Kuo and M.M. Kenty, 1997. Seed protein and its relationship to soluble sugars in soybean. Crop Sci., 37: 770-773.

Illahi, I. and M. Jabeen, 1992. Tissue culture studies for micropropagation and extraction of essential oils from ***Zingiber officinale*** Rosc. Pak. J. Bot., 24: 54-59.

Jana, P.K., S. Shatak, A. Barik, B.C. Biswas, G. Sounda and A.K. Mukherjee, 1990. Response of summer groundnut to phosphorus and potassium. Ind. J. Agron., 35: 137-143.

Jayapaul, P. and V. Ganesaraja, 1990. Studies on response of soybean varieties to nitrogen and phosphorus. Ind. J. Agron., 35: 329-330.

Jefing, Y., D.F. Herridge, M.B. Peoples and B. Rerkasem, 1992. Effects of N fertilization on N_2 fixation and N balances of soybean grown after lowland rice. Plant and Soil, 147: 235-242.

Khan, A., 1990. Nutritional significance and structure of soy carbohydrates-An overview. Sarhad J. Agric., 6: 335-342.

Khushwaha, H.S. and A.S. Chandel, 1997. Effect of nitrogen on yield, yield attributes and quality of soybean (***Glycine max*** L.) intercropped with cereals in foot hills of Uttar Pradesh. Ind. J. Agron., 42: 409-413.

Ladha, J.K., M.G. Kundu, A. Coppenolle, M.B. Peoples and P.J. Dart, 1996. Legume productivity and soil nitrogen dynamics in lowland rice-based cropping systems. Soil Sci. Soc. Am. J., 60: 183-192.

Liu, K.S., F. Orthoefer and E.A. Brown, 1995. Association of seed size with genotypic variation in the chemical constituents of soybeans. J. Amer. Oil Chem. Soc., 72: 189-192.

Malik, C.P. and A.K. Srivastava, 1979. Textbook of Plant Physiology. Kalyani Publishers, New Dehli, India, pp: 733.

Pourdavai, H. and F. Yousefi, 1993. The effect of Nitragin ''Commercial form of bacteria'' on grain yield and other parameters of soybeans. Seed and Plant, 8: 21-27.

Quattava, S. and D.B. Weaver, 1994. Effect of growth habit and agronomic characteristics of late planted soybean. Crop Sci., 34: 870-873.

Shah, S.M., M. Aslam, S. Shafiq and M.A. Hayat, 1999. Effect of sowing dates and row spacing on soybean yields and yield components in autumn under rainfed conditions. Pak. J. Bot., 31: 103-108.

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 Production and Environment, 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. Plant Nutri., 21: 2065-2075.

Tinius, C.N., J.W. Burton and T.E. Carter, 1993. Recurrent selection for seed size in soybean:3. Indirect effects on seed composition. Crop Sci., 33: 959-962.

Wilcox, J.R. and G.D. Zhang, 1997. Relationship between seed yields and seed protein in determinate and indeterminate soybean populations. Crop Sci., 37: 361-364.

Wilcox, J.R., 1998. Increasing seed protein in soybean with eight cycles of recurrent selection. Crop Sci., 38: 1536-1540.