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Effect of Fertilizer, Inoculation and Sowing Time on the Chemical Composition of Field Grown Soybean Seeds

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Abstract: The study was conducted on two different sowing dates in response to various level of added N fertilizer on the chemical components (viz., soluble protein, oil contents, soluble sugar, and starch content) of mature soybean cv. Williams-82 seeds. Fertilizer treatments were applied to both non-inoculated (non-inoc) and inoculated (inoc) crop. Results showed that fertilizer treatments in general significantly ($P<0.05$) reduced the protein content (407.5 to 364.6 g kg^{-1}) of late plantings (LP), but inconsistently increased (396.4 to 427.6 g kg^{-1}) in early plantings (EP). While inconsistent reduction in oil content (188.7 to 162.0 g kg^{-1}) was noted in both plantings. However, soluble sugars (48.39 to 63.32 g kg^{-1}) and starch (79.4 to 132.58 g kg^{-1}) were significantly increased in LP, but reverse was true in case of EP. By comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general significantly ($P<0.05$) increased the soluble sugars (20.66 to 23.95 g kg^{-1}) and starch content (113.5 to 146.0 g kg^{-1}) of EP, but reverse was found in LP seeds. While in case of EP, seed proteins generally respond non-significantly, and oil contents were negatively influenced by inoculation. However, on the basis of grand mean values, data suggested that EP seeds were chemically sound as compared with LP seeds. Based on correlation coefficient (r) studies, it can be concluded that protein content of LP seeds exhibited highly significant ($P<0.01$) positive association with oil contents (0.501) without any concomitant loss in their grain yield, whereas protein content of EP seeds exhibited highly significant ($P<0.01$) positive correlation with grain yield (0.683) without any concomitant loss in their oil contents.

Key words: Soybean, fertilizer, inoculation, sowing time, protein, oil, soluble sugars, starch, correlation

Introduction

Soybean (*Glycine max* L.) is one of the most important oil and protein crops in the world and can grow successfully throughout the country. 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; Shah *et al.*, 1999). The domestic production of edible oil in the country is hardly meeting 30-40 % of the total requirement. The present total requirement of edible oil is more than 2.0 million tones. The soybean seed contains 40-42 % good quality proteins 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. So it is highly desirable in human diet (Anonymous, 1994; Aslam *et al.*, 1995; Haq *et al.*, 2002). Even though the mineral composition of soybean is fairly good, but it is notorious for its low carbohydrate contents. Soybeans have almost no starch, but have enough oligosaccharides and crude fiber. The oligosaccharides (viz., raffinose, stachyose, and verbascose) are the water-soluble saccharides, and the causative factors for the flatulence character of soybeans. These saccharides can't be digested by human small intestine as the galactosidase enzyme, which breaks down these carbohydrates, are lacking in human digestive system. Research revealed that almost one third (35 %) of the whole soybean is carbohydrates, including polysaccharides, stachyose, raffinose, and sucrose. Khan (1990) reported that four cultivars of soybean (on moisture free basis) contain an average of 7.53 sucrose, 2.42 reducing sugars and 4.32 % starch. While Homowitz *et al.* (1972) stated that soybean contains 5.6-10.9 total sugar, 2.5-8.2 sucrose, 0.1-0.9 raffinose, and 1.4-4.1 % stachyose.

Several researchers revealed different responses of soybean seed protein and oil contents in relation to various level of added NPK fertilizers. Babich and Petrichenko (1992ab) observed that NPK fertilizer increased the seed amino acid content, but showed little effect on the amino acid composition of the protein. Khushwaha and Chandel (1997) recorded that soybean seed protein content was not significantly affected by nitrogen, although it improved with added nitrogen fertilizer. However, Sugimoto *et al.* (1998) stated that application of N fertilizer led faster accumulation of oil, a decrease in total protein, and some amino acids of developing soybean seeds.

Although seed yield and protein content are both heritable traits, but increasing seed protein concentration, without a concomitant loss in either seed oil or seed yield, should increase the value and quality of soybean seeds. However, 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 it's composition are often found significant.

The study was therefore mainly designed to determine the effect of added N fertilizer (without and with inoculums), and sowing time on the protein, oil, soluble sugars, and starch content of mature soybean seeds. The study was also initiated to furnish the information on the nature of association among different chemical components, and also with their yield to choose suitable selection criteria for predicting the quality and quantity of grain yield in soybean cv. Williams-82.

Materials and Methods

Two-year field experiments on soybean *Glycine max* L. Merill cv. Williams-82 were carried out during the 1st week of July, 1996 (late sowing) and June, 1997 (early sowing) in Agricultural Research Institute (ARI), Quetta, respectively. Seven different treatments of fertilizer were applied to both non-inoculated and inoculated set of experiments:

T_1: was kept as control (check); T_2: contained 60 + 30 kg P_2O_5 ha^{-1} and K_2O ha^{-1}, respectively. Whereas, 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 field layout, kind, source, and time of fertilizer application as well as the inoculums used were followed by Achakzai *et al.* (2002a). Finally the mature dry seed of all treated plants were separately collected when the plants attained their physiological maturity with complete senescence of leaves and yellow brown coloration. Ten-gram seed of each sample was then ground separately in a grinder and sieved through mesh No. 60 (Johnson & Firth Brown Ltd. London), and used for the determination of the following chemical components:

Soluble protein analyses (g kg^{-1}): Before soluble protein analyses, 0.2 g air-dried defatted seed powder of each sample was separately homogenized in 20 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). Each sample was centrifuged at 5,000 rpm using IEC B-20A Centrifuge (Damon/IEC Division) for 20 minutes, and filtered through Whatmann filter paper. Soluble protein was then determined by using Biuret reagent, and the procedure followed as described by Harris and Angal (1989). 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).

Oil content (g kg^{-1}): Oil contents were determined following the procedure described by Illahi and Jabeen (1992). Air-dried soybean powder (3.0 g) was homogenized in 27 ml diethyl ether at room temperature for 24 hours with continuous shaking @ 250 rpm, filtered through Whatmann filter paper No. 40. The suspension was then evaporated at 70 ^{0}C using water bath, and oil contents were finally calculated as under:

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

Soluble sugars and starch (g kg^{-1}): Soluble sugars and starch were determined by Malik and Srivastava (1985) 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 h and filtered through Whatmann filter paper. The residue was re-suspended in ethanol to remove all the traces of soluble sugars, and filtered it again. The filtrate was then diluted to 100 ml with deionized 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 hour and filtered. This process was repeated twice and the volume of the filtrate was also adjusted to 100 ml with water and then 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 minutes. After cooling, the absorbance was monitored at 625 nm using the same spectrophotometer. Total soluble sugars or starch were then calculated as under:

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

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), and least significance difference (LSD) under factorial randomized complete block design (RCBD). Simple correlation coefficient (r) studies were also worked out for the aforesaid chemical components and yield plot^{-1} following the Fisher and Yates (1953) statistical Table of significance.

Results and Discussion

Protein: Data (Table 1) showed that fertilizer treatments in general significantly ($P<0.05$) reduced the protein content of LP (except T_2), but inconsistently increased in EP, and mathematically a maximum protein content was recorded in T_7, T_6 and T_2 (427.6, 427.4 and also 427.4 g kg^{-1}) respectively. These findings are in accordance with the results obtained by Jayapaul and Ganesaraja (1990) and Achakzai *et al*. (2002b). Protein content of seeds were increased by added N fertilizer, but this increase was statistically found non-significant (Khushwaha and Chandel, 1997; Sugimoto *et al*., 1998). Result also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general did not significantly affect the seed protein content of EP except T_5 and T_7 (420.9 and 427.6 g kg^{-1}), but reverse was true in case of LP. These findings are also in conformity with the results obtained by Achakzai *et al*. (2002b), but are in contradiction with those of Pourdavi and Yousefi (1993) and Gretzmacher *et al*. (1994). On the basis of marginal mean values, the inoculation effect was recorded as 5.08 and 0.44 % greater in LP and EP when compared with their respective non-inoculated treatments. However on the basis of grand mean values, the EP seed produced 3.94 % greater protein content over LP seeds, which clearly signifies the importance of early planting over late plantings.

Oil contents: Data (Table 1) showed that fertilizer treatments in general did significantly but inconsistently reduced the oil content of both plantings when compared to their respective check (T_1). However, a maximum significant increase was noted in T_4 (216.6 g kg^{-1}) of EP and T_2 (177.7 g kg^{-1}) of LP respectively. This is to some extent in conformity with Achakzai *et al*. (2002b), but in contradiction with the results obtained by Jana *et al*. (1990) and Sugimoto *et al*. (1998). Results also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation in general significantly but adversely affected the oil content of EP seeds, whereas, non-significant effects were found in LP seeds. These findings are conflicting with the results obtained by Pourdavi and Yousefi (1993). This might be due to deceiving nodulation as already explained by Achakzai *et al*. (2002ab). While on the basis of marginal mean values, the inoculation effects are recorded as 1.46 % greater in LP and 1.47 % lesser in EP when compared with their respective non-inoculated treatments. However, on the basis of grand mean values, EP produced 10.32 % greater oil content over LP seeds. This enhancement in oil concentration could be mainly owe to early sowings, and plants would have got higher seed fill temperatures during the course of seed development, but these findings are not in agreement with Mark *et al*. (1997).

Soluble sugars: Data (Table 1) exhibited that lower doses of fertilizer (up to T_5) significantly ($P<0.05$) and positively influenced the soluble sugar of LP seeds. While reverse was true in case of EP seeds (except T_2) when compared with treatments not receiving fertilizer (T_1). Statistically a maximum significant amount of soluble sugar was recorded in T_2 (20.65 g kg^{-1}) of EP and T_4 (63.32 g kg^{-1}) of LP seeds. Though, no extensive study has been carried out on the effect of added N fertilizer on the soluble sugar of mature soybean seeds. However, Khan (1990) and Hartwig *et al*. (1997) reported that even-though the mineral composition of soybean is fairly good, but it is notorious for it's low carbohydrate content, having an average of 9.95 % soluble sugars. While in present study the mean minimum and mean maximum soluble sugars of non-inoculated fertilized seed are ranges from 2.06 to 6.33 %, which are far lesser than those reported by aforementioned workers. Results also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly and positively affected the soluble sugar of EP, but reverse was true in case of LP seeds. The mean minimum and mean maximum value of inoculated and non-inoculated fertilized treatments are ranges from 1.74 to 5.44 %, which are also far lesser than those reported by Khan (1990) and Hartwig *et al*. (1997). Whereas on the basis of marginal mean values, the inoculation effect was recorded as 26.13 % greater in EP, and 10.32 % lesser in LP seeds when compared with their respective non-inoculated treatments. However, on the basis of grand mean values, EP seed produced nearly 1.5 fold lesser soluble sugars over LP seeds. It is therefore, can be safely concluded that the decreased level of soluble sugars in EP could increase the quality of soybean seeds. Because the soluble sugars i.e., oligosaccharides (like raffinose, stachyose, and verbascose) are the causative factors for the flatulence character of soybeans. These saccharides can't be digested by human small intestine.

Starch contents: Data (Table 1) showed that fertilizer treatments in general significantly increased the starch content of LP (except

Table 1: Effect of various level of added fertilizer without inoculums (non-inoc) and with inoculums (inoc) on the chemical components of mature soybean cv. Williams-82 seeds grown on two different sowing dates

	Chemical components (g kg^{-1})							
	Soluble protein		Oil contents		Soluble sugars		Starch contents	
Treatments	LP	EP	LP	EP	LP	EP	LP	EP
T_1 (non-inoc)	407.5d	396.4f	176.4b	188.7cd	48.39e	18.72g	79.4d	112.30h
(inoc)	416.2c	398.2f	177.1ab	182.1gh	45.67g	20.66f	77.41e	113.50g
T_2 (non-inoc)	443.9a	427.4b	177.7a	186.1gh	53.39d	20.65f	77.27e	100.80j
(inoc)	445.3a	427.5b	177.8a	186.4gh	47.54f	22.62c	75.82f	107.30i
T_3 (non-inoc)	364.6i	408.1e	162.0g	191.0c	55.32b	16.34i	81.34c	93.24k
(inoc)	377.4g	408.6e	177.8a	185.4ef	48.49e	23.95a	61.53j	121.50d
T_4 (non-inoc)	373.2h	417.9d	166.5c	216.6a	63.32a	18.22h	104.40b	88.10l
(inoc)	384.8f	417.2d	166.5c	200.8b	54.42c	23.09b	63.21i	118.6e0
T_5 (non-inoc)	377.7g	417.2d	164.4de	183.3fg	54.28c	18.02h	103.50b	115.90f
(inoc)	397.1e	420.9c	164.7d	182.5gh	48.46e	21.82d	62.83i	128.30b
T_6 (non-inoc)	393.6e	427.4b	163.4f	181.7gh	43.71j	16.63i	132.50a	108.10i
(inoc)	427.2b	427.5b	163.5ef	180.6gh	42.44j	22.73c	59.65k	123.70c
T_7 (non-inoc)	406.6d	427.6b	166.9c	180.3h	43.06i	15.18j	72.10g	114.40g
(inoc)	417.7c	433.9a	167.1c	170.9i	40.62k	21.21e	69.64h	146.00a
LSD (0.05) value	3.732	3.575	0.8574	2.868	0.5814	0.3095	1.255	1.136
CV (%)	0.55	0.51	0.30	0.91	0.70	0.92	0.93	0.59
MM (non-inoc)	389.59	417.29	168.19	186.81	51.64	17.82	92.88	104.68
MM (inoc)	409.39	419.11	170.64	184.10	46.81	22.30	67.16	122.70
Grand Means	402.343	418.195	169.410	186.889	49.222	19.898	80.023	113.694

Mean values in a column followed by different letters differ significantly at $P<0.05$ LP = Late plantings EP = Early plantings
CV = Coefficient of variance, MM = Marginal mean, Non-inoc = Non-inoculated, Inoc = Inoculated
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 2: Correlation coefficient (r) studies of various chemical components of field-grown mature soybean seeds in response to various level of added N fertilizer (without and with inoculums) at two different sowing dates

Chemical components (g kg^{-1})	Protein LP EP	Oil content LP EP	Soluble sugars LP EP	Starch content LP EP	Grain yield LP EP
Protein					
LP	1.000				
EP	1.000				
Oil content					
LP	0.501**	1.000			
EP	-0.279NS	1.000			
Soluble sugars					
LP	-0.507**	-0.064NS	1.000		
EP	0.042NS	-0.063NS	1.000		
Starch content					
LP	-0.290NS	-0.272NS	0.242NS	1.000	
EP	0.273NS	-0.679**	0.464*	1.000	
Grain yield					
LP	0.138NS	-0.199NS	-0.108NS	-0.99NS	1.000
EP	0.683**	-0.247NS	-0.211NS	0.331NS	1.000

*: $P<0.05$ **: $P<0.01$ NS = Non-significant

T_2 and T_7), but reverse was true in case of EP seeds (except T_5 and T_7). Results also showed that by comparing the inoc with non-inoc treatments in particular doses of fertilizer, inoculation significantly increased the starch content of EP, but reverse was found in case of LP seeds. Research revealed that soybean seeds contained an average of 4.32 % starch. In present studies the mean value of starch content in non-inoc and inoc treatments are ranges from 7.21 to 13.25 % and 6.15 to 14.60 % respectively, which are far greater than that reported by Khan (1990). On the basis of marginal mean values, the inoculation effect was recorded as 17.21 % greater in EP and 27.69 % lesser in LP as compared with their respective non-inoc treatments. However, on the basis of grand mean values, the EP produced 42.08 % greater starch content over LP seeds. This surplus response of starch by inoculation and fertilization is therefore clearly suggested that planting date was the principal cause. There are other climatic factors like ETp (potential evapotranspiration) and RH (relative humidity), which might have also contributed to this differential response. Similar responses are also noted by Achakzai *et al*. (2002ab) in growth and yield studies of the same crop. Therefore, it can be safely concluded that on account of surplus amount of starch content and low level of soluble sugars in EP seeds could increase the value and quality of soybean, which could be used as a source of energy in human diet and poultry feed without creating any digestive disturbances.

Correlation studies: The correlation coefficient (r) studies revealed that soluble proteins of LP seed were highly significantly ($P<0.01$) and positively correlated with oil contents, but negatively with soluble sugars, and insignificantly with grain yield (Table 2). While in case of EP they exhibited highly significant positive (0.683) association only with their respective yield. It was also noted that starch content of EP did exhibit highly significant negative (-0.679) correlation with oil contents, but slightly positive (0.464) with soluble sugars. While the remaining chemical components respond non-significantly. Studies revealed that soybean meal, containing the protein has been the more valuable component of the seed. 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, present findings of both plantings are strongly in support of this idea, and are in agreement with the results obtained by Achakzai *et al.* (2002b), and in disagreement with the results described by Wilcox and Zhang (1997) and Wicox (1998). Several factors including usable fertilizer, sowing dates and N fixing rates may affect the relationship between seed protein content and grain yield. The present findings regarding correlation of protein with yield of EP could be attributed to cumulative effects of early sowing and solubilization of added fertilizers, which are also in conformity with some others (Imsande, 1989; Tinius *et al.*, 1993).

Therefore, in this study it can be concluded that for getting high concentration of protein and grain yield without disturbing their oil contents, early sowing with added fertilizer was choose. While, for getting high protein and oil contents without any concomitant loss in their grain yield, then to choose late sowing with added fertilizer. In conclusion, based on the quality of seeds, the EP seeds of soybean are comparatively better than that of LP seeds.

References

Achakzai, A.K.K., S.A. Kayani, S. Jehan, M.A. Wahid and S.H. Shah, 2002a. 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.

Achakzai, A.K.K., S.A. Kayani, M. Yaqoob and A. Nabi, 2002b. Effects of fertilizer and inoculation on the chemical composition of soybean cv. Williams-82 seeds in pot culture experiments. Pak. J. Bot., (submitted).

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

Aslam, M., S.M. Mirza, S.M. Shah, N. Javed and Naeemullah, 1995. New early maturing and high yielding soybean varieties. Crop Production Bulletin, No.4, pp: 1-11.

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.

Haq, I., I. Hussain, A.R. Khan, M. Sajid and S. Khan, 2002. Soybean genotypic response in Abbottabad. Asian J. Pl. Sci., 1: 418-419.

Harris, E.L.V. and S. Angal, 1989. Protein Purification - A Practical Approach. 1st edition, 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.

Homowitz, T., F.I. Collins, J. Panczner and W.M. Walker, 1972. Relationship between the content of oil, protein and sugar in soybean seed. Agron. J., 64: 613-616.

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.

Imsande, J., 1989. Rapid dinitrogen fixation during soybean pod fill enhances net photosynthetic output and seed yield: A new perspective. Agron. J., 81: 549-556.

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

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.

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

Malik, C.P. and A.K. Srivastava, 1985. Textbook of Plant Physiology. Kalyani Publishers, New Delhi, India.

Mark, V.K., C.S. Colleen, L.J. Grabau, C.T. MacKown and D.F. Hildebrand, 1997. Early maturing soybean cropping system:III. Protein and oil content and oil composition. Agron. J., 89: 464-469.

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 rain fed 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, UK, pp: 481.

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. Nutr., 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.