Effect of Sulphur and Nitrogen Application on Growth Characteristics, Seed and Oil Yields of Soybean Cultivars

Arshad Jamal*,, Inayat Saleem Fazli**, Saif Ahmad**, Malik Zainul Abdin**, and Song Joong Yun*,†**

*Division of Biological Resources Science, College of Agriculture and Life Science, Chonbuk National University, Jeonju, 561-756, South Korea.

**Department of Biotechnology, Hamdard University, Hamdard Nagar, New Delhi- 110062, India.

ABSTRACT : A field experiment was conducted to assess the growth characteristics, seed and oil yield of two cultivars of soybean (*G max* (L.) Merr.) cv. PK-416 (V_1) and cv. PK-1024 (V_2) in relation to sulphur and nitrogen nutrition. Six combinations (T_1-T_6) of two levels of sulphur (0 and 40 kg ha^{-1}) and two levels of nitrogen (23.5 and 43.5 kg ha^{-1}) were applied to the two soybean cultivars as nutrients. Results indicated significant effect of sulphur and nitrogen, when applied together, on the growth characteristics, yield components, and seed and oil yield. Maximum response was observed with treatment T_6 (having 40 kg S and 43.5 kg N ha^{-1}). Seed and Oil yields were increased 90 and 102% in V_1, and 104 and 123% in V_2, respectively as compared to the control i.e. T_1 (having 0 kg S and 23.5 kg N ha^{-1}). Positive responses of S and N interaction on leaf area index, leaf area duration, crop growth rate and biomass production were also observed. The results obtained in these experiments clearly suggest that balanced and judicious application of nitrogen and sulphur can improve both seed and oil yield of soybean cultivars by enhancing their growth.

Keywords: *Glycine max* (L.) Merr., nitrogen, oil yield, seed yield, sulphur

Sulphur (S) plays a pivotal role in various plant growth and development processes being a constituent of S containing amino acids, cystine and methionine, and other metabolites viz., glutathione and phytochelators. The latter contribute to stress repair and amelioration of heavy metals. Besides, a number of S-containing components are biologically active and, thus, a source for use as medicinal value.

The most important constraints to crop growth are those caused by shortage of plant nutrients. S is increasingly being recognized as the fourth major plant nutrient after nitrogen, phosphorus and potassium. The level of S in the soil is one of the critical factors determining the growth and yield of the plants (Lakkineni & Abrol 1994). Recently, widespread deficiency of S in the soil of crop fields has been noticed in many parts of India (Tandan, 1991).

Sufficient amount of S reserves are available globally which could be used for agricultural purposes. The S deficiency is, however, an important nutrient disorder in agricultural production in all continents. Besides a decrease in crop productivity (Scherer, 2001) and negative impact on crop quality (Abdin *et al.*, 2003a; Abdin *et al.*, 2003b) a higher susceptibility of crops to certain diseases was observed in S-deficient soils (Schnug *et al.*, 1995).

S is required along with nitrogen (N) in the synthesis of proteins and enzymes (Zhao *et al.*, 1999). It is also implicated in oil biosynthesis (Fazli *et al.*, 2005). The role of S in the seed production of soybean has also been reported by several investigators (Dubey & Billore 1995; Fontanive *et al.*, 1996; Shrivastava *et al.*, 2000). But these data are insufficient to provide a basis for evolving a management technology of S application with appropriate amount of N to optimize N-assimilation efficiency, and seed as well as oil yield of soybean. In this experiment, therefore, an attempt was made to evolve appropriate technology of S and N application for optimum growth, seed and oil yield of soybean.

MATERIALS AND METHODS

A field experiment, employing randomized block design, was conducted to study the interactive effect of S and N on growth characteristics, seed and oil yield of non-nodulating soybean (*Glycine max* L. Merr. cv. PK-416, V_1 and cv. PK-1024, V_2). The cultivars were grown on sandy loam soil at the experimental field of Hamdard University, India. The S content in the soil was 20 ppm.

The treatments consisted of six combinations of two levels of S (0 and 40 kg ha^{-1}) and two levels of N (23.5 and 43.5 kg ha^{-1}) : 0 S + 23.5 kg N ha^{1} (T_1); 0 S + 23.5+20 kg N ha^{-1} (T_2); 40 S + 23.5 kg N ha^{-1} (T_3); 40 S + 23.5+20 kg N ha^{-1} (T_4); 20+20 S + 23.5 kg N ha^{-1} (T_5); 20+20 S + 23.5+20 kg N ha^{-1} (T_6). N and S were given in split applications (first dose at the time of sowing and second at 35 days after sowing). Each treatment had three replications. The plot size

†Corresponding author: (Phone) +82-63-270-2508 (E-mail) sjyun@chonbuk.ac.kr <Received December 1, 2005>

was 9 m^2 (3 × 3 m). Phosphorous and potassium were applied to all the plots as basal dressings at the rate of 60 and 40 kg ha^{-1}each. The source of N, phosphorous, potassium and S were urea, diammonium phosphate, murate of potash and gypsum, respectively. Irrigation was applied as per requirement of the crop. By regular weeding operations, the crop was kept free from weeds. At two weeks after sowing, seedlings were thinned to keep an intrarow spacing of 45 cm and plant to plant distance of 5 cm.

An area (1 m^2) of each plot was earmarked for the purpose of harvest, analysis of seed yield and its components. The remaining rows (except the border rows) were used for taking plant samples. The sampling was done at 30, 45, 60, 75, 90, 105 days after sowing (DAS) and at harvest. Three plants were taken from each plot randomly. The samples were cut at root-shoot junction, brought to the laboratory in moist polythene bags and immediately weighed. Leaves were separated from the stem and the leaf area was measured using a leaf area meter (Model *LI COR* 3000, USA). Stems were cut into small pieces. The stem and leaf samples were dried separately in an oven at 80 °C for 72 h. The biomass, seed yield and yield components were determined at harvest from an area of 1 m^2 from each plot. Oil content of seed was measured by rapid gravimetric method developed

Table 1. Effect of S and N on seed yield, biological yield and harvest index of soybean (*Glycine max* (L.) Merr.) cultivars.

Treatment	Seed yield (kg / ha)	Biological yield (kg / ha)	Harvest index (%)
***Glycine max* (L.) Merr. cv PK- 416 (V1)**			
T_1	1798	6314	28.47
T_2	2077	7028	29.55
T_3	2536	7480	33.90
T_4	3380	9769	34.59
T_5	3107	9095	34.16
T_6	3837	10153	37.79
***Glycine max* (L.) Merr. cv PK-1024 (V2)**			
T_1	1966	7002	28.07
T_2	2186	7570	28.87
T_3	2742	8328	32.92
T_4	3735	10267	36.38
T_5	3443	9649	35.67
T_6	3967	10415	38.08
LSD (0.05)			
Cultivars (V)	54.500	130.100	0.262
Treatments (T)	94.400	225.350	0.454
V × T	133.500	318.690	0.642

T_1 = 0S + 23.5N; T_2 = 0S + 23.5 + 20N; T_3 = 40S + 23.5N; T_4 = 40S + 23.5 + 20N; T_5 = 20 + 20S + 23.5N; T_6 = 20 + 20S + 23.5 + 20N; LSD = Least Significant Difference

Table 2. Effect of S and N on number of pod $plant^{-1}$, number of seed pod^{-1} and 100-seed weight of soybean (*Glycine max* (L.) Merr.) cultivars.

Treatment	No of Pod $Plant^{-1}$	No.of Seed Pod^{-1}	100 Seed weight (g)
***Glycine max* (L.) Merr. cv PK- 416 (V1)**			
T_1	32.00	2.33	12.12
T_2	38.67	2.43	12.64
T_3	48.00	2.70	13.57
T_4	64.33	2.77	14.47
T_5	60.67	2.73	14.23
T_6	70.67	2.90	15.18
***Glycine max* (L.) Merr. cv PK-1024 (V2)**			
T_1	34.67	2.40	11.90
T_2	40.67	2.49	12.11
T_3	50.00	2.77	13.01
T_4	67.00	2.83	14.18
T_5	65.33	2.79	13.93
T_6	74.00	2.93	14.91
LSD (0.05)			
Cultivars (V)	1.091	0.059	0.035
Treatments (T)	1.890	0.103	0.060
V × T	2.673	0.146	0.085

T_1 = 0S + 23.5N; T_2 = 0S + 23.5 + 20N; T_3 = 40S + 23.5N; T_4 = 40S + 23.5 + 20N; T_5 = 20 + 20S + 23.5N; T_6 = 20 + 20S + 23.5 + 20N; LSD = Least Significant Difference

Table 3. Effect of S and N on seed yield, oil content and oil yield of soybean (*Glycine max* (L.) Merr.) cultivars.

Treatment	Seed yield (kg / ha)	Oil content (%)	Oil yield (kg / ha)
***Glycine max* (L.) Merr. cv PK- 416 (V_1)**			
T_1	1798	21.30	383
T_2	2077	21.70	452
T_3	2536	22.50	570
T_4	3380	23.27	786
T_5	3107	22.77	707
T_6	3837	23.53	903
***Glycine max* (L.) Merr. cv PK-1024 (V_2)**			
T_1	1966	20.20	396
T_2	2186	20.33	444
T_3	2742	21.47	588
T_4	3735	22.63	845
T_5	3443	22.32	767
T_6	3967	22.93	909
LSD (0.05)			
Cultivars (V)	54.500	0.120	12.900
Treatments (T)	94.400	0.208	22.340
V × T	133.500	0.294	31.590

T_1 = 0S + 23.5N; T_2 = 0S + 23.5 + 20N; T_3 = 40S + 23.5N; T_4 = 40S + 23.5 + 20N; T_5 = 20 + 20S + 23.5N; T_6 = 20 + 20S + 23.5 + 20N; LSD = Least Significant Difference by Kartha & Sethi (1957). Oil yield was calculated on the basis of oil percentage and seed yield. The statistical analysis was done following the method of Nageswar (1983). Harvest index was calculated by the following equation given by Donald & Hamblin (1976): Harvest index = [Seed yield (g m^{-2}) / Biological yield (g m^{-2})] × 100.

RESULTS AND DISCUSSION

It was observed that all the parameters studied were affected significantly by the combined application of S and N fertilizers. It was evident from Table 1 to 3 that treatment T_6 (20 + 20 S and 23.5 + 20 kg N ha^{-1}) proved optimal for most of the yield parameters, including seed and oil yield in the two cultivars. As compared with the treatment T_1 (having only 23.47 kg N ha^{-1}), seed yield was 113.4 % and 101.7 %, biological yield 60.80% and 48.74 %, and harvest index 32.73% and 35.66% higher in V_1 and V_2, respectively with treatment T_6 (Table 1), the major contributing parameters being number of pods per plant (120.8% inV_1 and 113.4% in V_2), number of seeds per pod (24.4% inV_1 and 22.0% in V_2) and 100-seed weight (25.2% inV_1 and 25.2% in V_2) (Table 2). Oil yield per hectare was accordingly increased by 135.7 % and 129.5% in V_1 and V_2, respectively with T_6 treatment (Table 3). Similar trends in the seed and oil yield were observed in rapeseed-mustard also with the combined application of S and N (Ahmad *et al.* 1998).

Leaf area index, leaf area duration and biomass accumula-

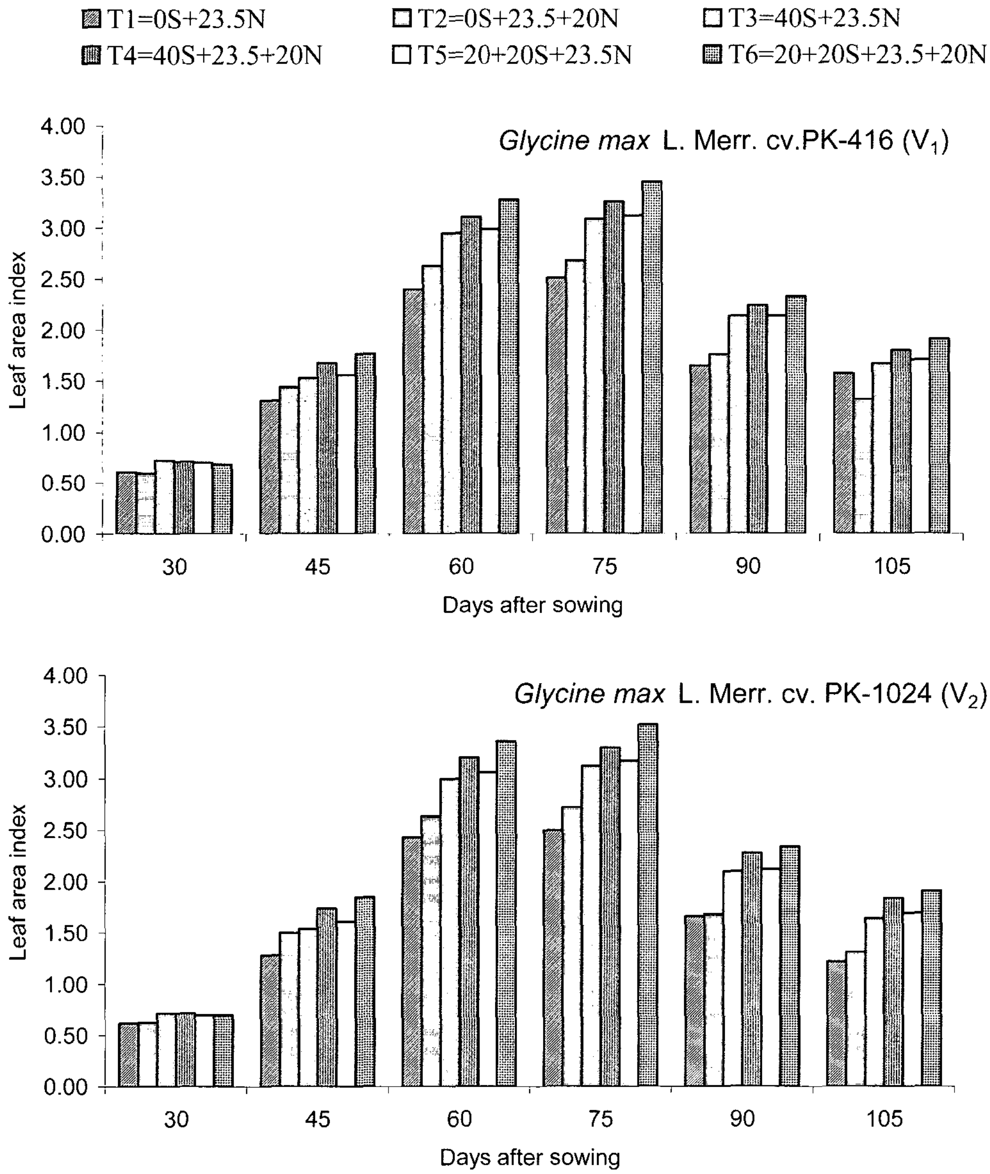

Fig. 1. Effect of S and N on leaf area index of soybean at various growth stages.

tion at various growth stages were found to be higher with treatments having S and N in combination than the treatment having N alone. The leaf area index attained a peak at 75 DAS in both cultivars, and declined thereafter in all treatments. The highest leaf area index was attained by both cultivars at treatment T_6 until 75 DAS (Fig. 1). The leaf area duration (LAD) was maximum at 60-75 DAS in both cultivars and declined thereafter in all treatments. The highest LAD was attained by both cultivars at treatment T_6 until 60-75 DAS (Fig. 2). Maximum biomass accumulation was observed in both cultivars with the treatment T_6 at 105 DAS (Fig. 3).

The high response of soybean cultivars to the treatment T_6 may be due to the balanced application of N and S. Since these nutrients are involved in the biosynthesis of proteins and many other important biomolecules, a balanced application of S and N enhanced their use efficiency in crop plants. Maximum seed and oil yield was obtained in rapeseed mustard only, when S and N applications were balanced (Ahmad *et al.*, 1998). Similarly, a strong coupling between S and N has been established through many studies in terms of dry matter and seed as well as oil yield in several crops (Abdin *et al.* 2003a, Abdin *et al.* 2003b, Ahmad *et al.* 1998, Fazli *et al.* 2005, Lakkineni & Abrol 1992, Zhao *et al.* 1999). Barney & Bush (1985), concluded that in tobacco there was apparent accumulation of N when S was limited and vice-

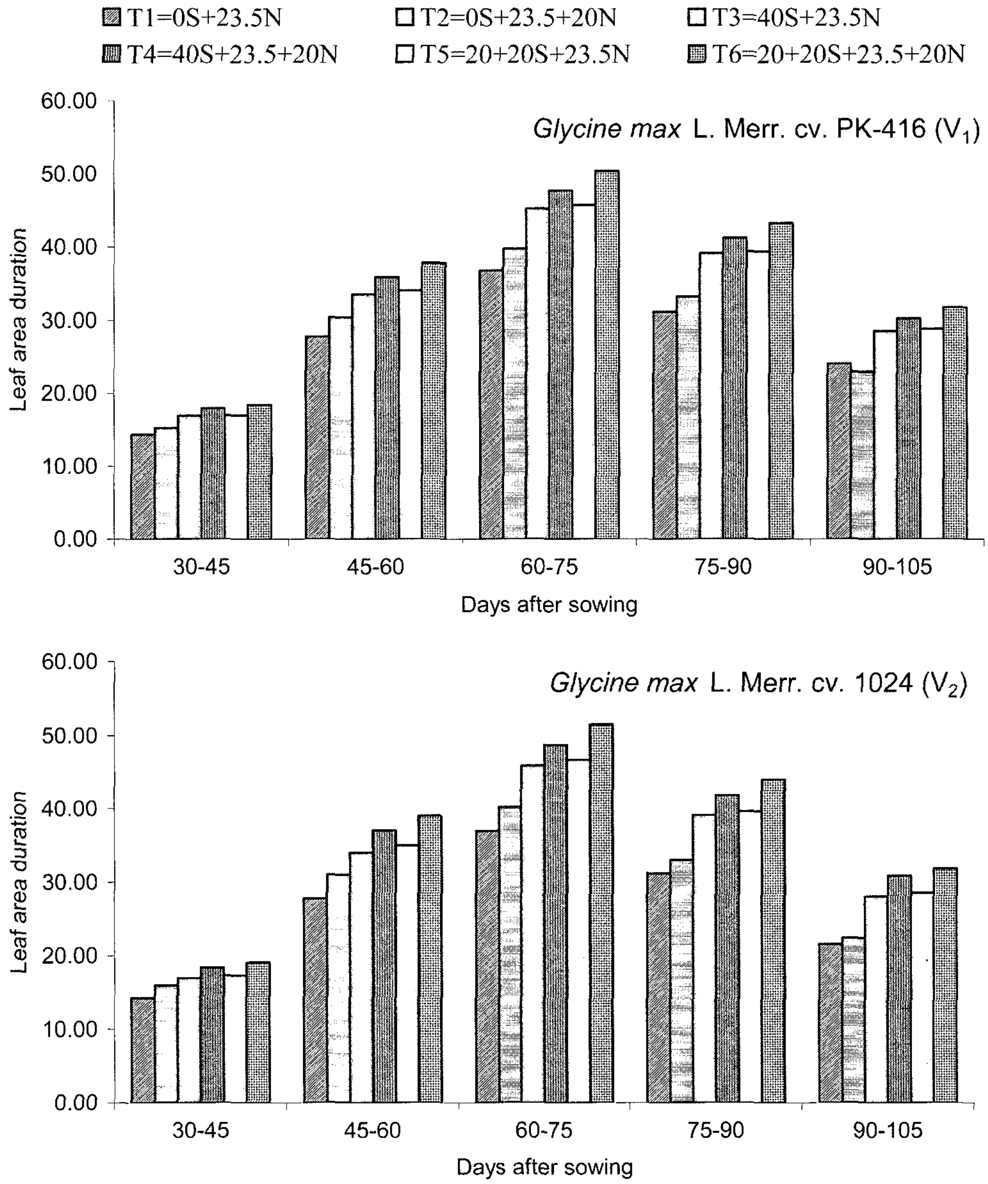

Fig. 2. Effect of S and N on leaf area duration of soybean at various growth stages.

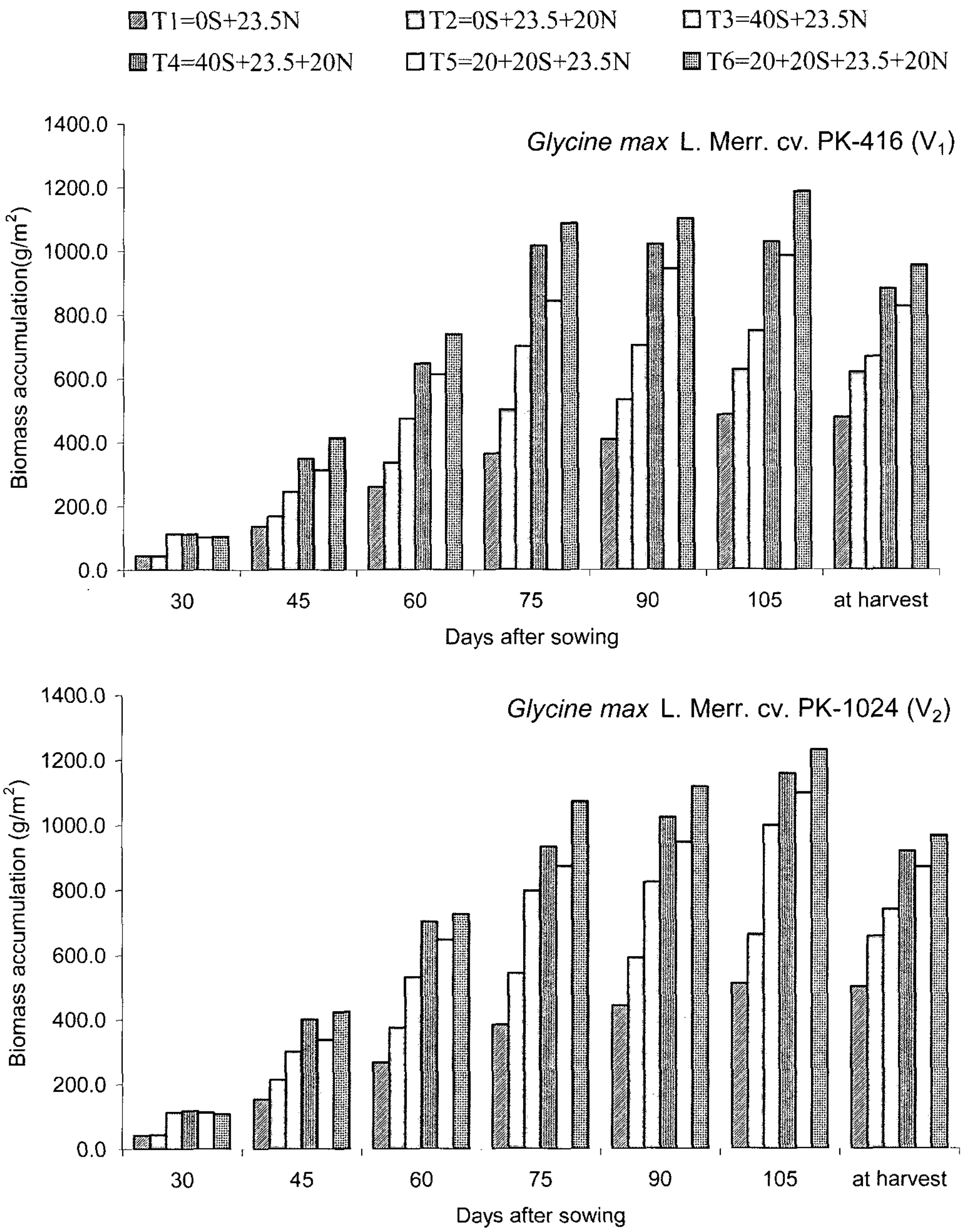

Fig. 3. Effect of S and N on biomass accumulation of soybean at various growth stages.

versa. Further they have demonstrated that the nutrients were used in protein synthesis, when they were supplied in adequate amounts. A shortage in the supply of S to the crops lowers the utilization of the available soil N, thereby increasing nitrate leaching (Lakkineni & Abrol 1994). O'Conner & Vartha (1969) observed that high doses of gypsum reduced the yield of hay when N status in soil was insufficient. Likewise, high doses of N created S deficiency (Eppendorfer, 1971). Highest N supply from deficient to adequate level resulted in a 2.4-fold increase in seed number per plant and a 2-fold increase in single seed weight, so that seed yield per plant increased 5-fold in sunflower (Hocking *et al.,* 1987). It has been established that for every 15 part of N in protien there is 1 part of S, which implies that the N:S ratio is fixed within a narrow range of 15:1. The N:S ratio in the whole plant in general is 20:1 (Cram, 1990).

Clarkson *et al.* (1989), demonstrated in barley plants that at the whole plant level the apparent matching of supply to demand is accompanied by an apparent linkage of SO_4^{2-} to NO_3^- uptake. The assimilatory pathways of these elements are considered functionally convergent (Filner, 1978). A positive role of sulphate in regulating nitrate reductase, an enzyme that catalyzes the rate-limiting step of nitrate assimilatory pathway was found by Friedrich & Schrader (1978). The role of N in the regulation of sulphate assimilation at the ATP sulphurylase step was observed by Smith (1975). The work of Barney & Bush (1985) showed that S availability has a role in regulating nitrate reductase, in addition to its role in regulating ATP sulphurylase. Moreover, N availability has a role in regulating ATP sulphurylase as well as in regulating nitrate reductase. The synthesis of cystein as a result of the incorporation of sulphide moiety into O-acetylserine appears to be the meeting point between N- and S-metabolism. Naturally occurring thiol compounds viz. cystein and glutathione were shown to influence to nitrate reductase activity in wheat and *Brassica* (Lakkineni & Abrol, 1992).

From the results obtained in this study it can be concluded that S and N interact at metabolic level in such a way that imbalance in their supply reduces the yield of crop. Hence, the inclusion of S (up to 20 kg/ha) in fertilizer recommendation for optimum soybean seed and oil yield in S-deficient soils is necessary, and S and N should be given in balanced doses to obtain optimum yield.

ACKNOWLEDGEMENTS

This work was supported by Technology Mission on Oilseeds, Pulses and Maize, Ministry of Agriculture and Cooperation and CSIR. The authors wish to thank Dr. S. C. Saxena, Scientist, Dept. of Agronomy, G. B. Pant University of Agriculture and Technology, Pantnagar, India, for providing soybean seeds to carry out this study. Song J. Yun was supported by Research Center for Industrial Development of BioFood Materials in Chonbuk National University, Jeonju, Korea. The center is designated as a Regional Research Center appointed by the Ministry of Commerce, Industry and Energy (MOCIE), Jeollabuk-do Provincial Government and Chonbuk National University.

REFERENCE

Abdin, M. Z., A. Ahmad, N. Khan, I. Khan, A. Jamal, and M. Iqbal. 2003a. Sulphur interaction with other nutrition. In: Y. P. Abrol and A. Ahmad (eds), Sulphur in plants, pp. 359-374. Kluwer Academic Publishers, Dordrecht.

Abdin, M. Z., N. Khan, I. Khan, M. Israr, and A. Jamal. 2003b. Nitrogen and sulphur interaction in relation to yield and quality attribute of rapeseed-mustard. Brassica 5 (3) : 35-41.

Ahmad, A., G Abraham, N. Gandotra, Y. P. Abrol, and M. Z. Abdin 1998. Interactive effect of nitrogen and sulphur on growth and yield of rapeseed-mustard (*Brassica juncea* [L.] Czern and Coss. and *Brassica campestris* L.). J. Agron. Crop. Sci. 181 : 193-199.

Barney, Jr. P. E. and L. P. Bush. 1985. Interaction of nitrate and sulphate reduction in tobacco.I. Influence of availability of nitrate and sulphate. J Plant Nutr. 8 : 507-515.

Clarkson, D. T., L. R. Saker, J. V. Purves, and R. B. Lee. 1989. Depration of nitrate and ammonium transport in barley plant with diminished sulphate status. Evidence of co-regulation of nitrogen and sulphate intake. J Expt Bot. 40 : 953-963.

Cram, W. J. 1990. Uptake and transport of sulphate. In: Rennenberg H (eds.) Sulphur Nutrition and Assimilation in Higher Plants, pp, 3-11 SPB Acadmic Publishing, The Hague, The Netherlands.

Donald, C. M. and J. Hamblin. 1976. The Biological yield and harvest index of cereals as agronomic and plant breeding criteria. Adv Agron. 28 : 361-405.

Dubey, S. K. and S. D. Billore 1995. Effect of level and source of sulphur on symbiotic and biometric parameters of soybean (*Glycine max*). Ind J Agric Sci. 65 : 140-144.

Eppendorfer, W. H. 1971. Effects of sulfur, nitrogen and phosphorous on amino acid composition of field beans (*Vicia faba*) and responses of the biological value of the seed protein and sulfur-amino acid content. J. of the Sci. of Food and Agric. 22 : 501- 505.

Fazli, I. S., M. Z. Abdin, A. Jamal, and S. Ahmad. 2005. Interactive effect of sulphur and nitrogen on lipid accumulation, acetyl-CoA concentration and acetyl-CoA carboxylase activity in developing seeds of oilseed crops (*Brassica campestris* L. and *Eruca sativa* Mill.). Plant Sci. 168 : 29-36.

Filner, P. 1978. Regulation of inorganic nitrogen and sulphur in cell suspension cultures. In: TA Thrpe (eds.), Proc 4th Intl Cong Plant Tissue Culture, pp. 437-442.

Fontanive, A. V., A. M. de la Horra, and M. Moretti 1996. Foliar analysis of sulphur in different soybean cultivar stages and its relation to yield. Commun Soil Sci Plant Anal. 27 : 179-186.

Friedrich, J. W. and L. E. Schrader 1978. Sulphur deprivation and nitrogen metabolism in maize seedlings. Plant Physiol. 61 : 900-903.

Hocking, P. J., P. J. Randall, and A. Pinkerton. 1987. Sulfur nutrition of sunflower (*Helianthus annus*) as affected by nitrogen supply. Effect on vegetative growth, development of yield components and seed yield and quality. Field Crop Res. 16 : 157-175.

Kartha, A. R. S. and A. S. Sethi. 1957. A cold percolation method for rapid gravimetric estimation of oil in small quantities of oil seeds. Ind. J. Agri.c Sci. 27 : 211-217.

Lakkineni, K. C. and Y. P. Abrol. 1992. Sulphur requirement of rapeseed-mustard, groundnut and wheat: A comparative assessment. J. Agron. Crop. Sci. 169 : 281-285.

Lakkineni, K. C. and Y. P. Abrol. 1994. Sulphur requirement of crop plants:Physiological Analysis. Fert. News 39 : 11-18.

Nageswar, R. G. 1983. Statistics for agricultural sciences. Oxford and IBH Publishing Co., Oxford.

O'Conner, K. F. and E. W. Vartha 1969. Responses of Grasses to Sulfur fertilizers. Plant and Soil. 60 : 451-459.

Scherer, H. W. 2001. Sulphur in crop production. Euro. J. Agron. 14 : 81-111.

Schnug E., E. Haneklaus, E. Booth, and K. C. Walker. 1995. Sulfur and stress resistance in oilseed rape. Proceeding of the 9th International Rapeseed congress, Vol. 1 of 4, pp. 229-231. Rapeseed Today and Tomarrow, Cambridge.

Shrivastava, U. K., R. L. Rajput, and M. L. Devedi. 2000. Response soybean-mustard cropping system to sulphur and biofertilizers on farmers field. Legume Res. 23 : 277-278.

Smith, I. K. 1975. Sulphate transport in cultured tobacco. Plant Physiol. 55 : 303-307.

Zhao, F. J., M. J. Hawkesford, and S. P. McGrath 1999. Sulfur assimilation and effects of yield and quality of wheat. J. Cereal. Sci. 30: 1-17.