Applied Biological Research 17 (1): 84-89; (2015) **SHORT COMMUNICATION**
DOI: 10.5958/0974-4517.2015.00012.9

EFFECT OF NITROGEN AND SULPHUR ON THEIR RATIO, SEED YIELD AND PROTEIN RELATED PARAMETERS IN DEVELOPING SOYBEAN

Anju Sharma*, Sucheta Sharma and B.S. Gill[1]

Department of Biochemistry, College of Basic Sciences and Humanities, [1]Department of Plant Breeding, College of Agriculture, Punjab Agricultural University, Ludhiana, Punjab – 141 004 (India)
*e-mail: *anju3sep@gmail.com*

(Received 14 May, 2014; accepted 2 October, 2014)

ABSTRACT

The present paper reports the effect of nitrogen and sulphur application on their ratio, seed yield and protein related parameters in soybean. A random block design experiment with four treatments [control, nitrogen (urea @ 31.25 kg N ha^{-1}) and sulphur (gypsum @ 20 kg S ha^{-1}) alone or in combination] was conducted at PAU, Ludhiana (India) during *kharif* season 2012 using soybean cultivar 'SL 525' as a test crop. Nitrogen and sulphur alone or in combination significantly increased seed yield and decreased N:S ratio in the developing soybean leaves, seeds, roots and stem. Total soluble proteins, free amino acids and S-containing amino acids (only in leaves) in developing leaves and seeds increased due to nitrogen and sulphur supply. Results suggested that nitrogen alone causes maximum protein accumulation and sulphur alone or in combination with nitrogen maximally increases free amino acids, S-containing amino acids, seed yield and decreases N:S ratio in soybean.

Key words: Nitrogen, N:S ratio, protein, soybean, sulphur, yield

INTRODUCTION

Soybean (*Glycine max* L. Merrill), miracle golden bean, is excellent source of edible protein and oil. The nutritional quality of soybean depends on nutrients availability to the plant. Nitrogen (N) and sulphur (S) are essential elements required for plant growth, high seed yield, as components of amino acids and proteins, cofactors of various enzymes, etc. Soybean fixes atmospheric N symbiotically but this N is insufficient to meet its requirement (Kumawat *et al.,* 2000; Duraisami and Mani, 2001). N and S mobilization from vegetative tissues to seed, their status in tissues and seeds and their distribution along main stem is a limiting factor for seed composition constituents, mainly for protein and amino acid profile. For seed composition, high N mobilization from vegetative tissue to seed is required. Seed N concentration is correlated with N availability within plants and the contribution of N remobilization to seed N accounts for 80-90% in soybean (Bellaloui and Gillen, 2010). Soybean mobilizes 65-79% of its vegetative N and 66-79% S to seeds (Vasilas *et al*., 1995) and both N and protein in vegetative tissues are important source of remobilizable S. In soybean, N and S assimilating pathways are very well coordinated and deficiency of any of these elements represses the other pathways (Kopriva and Rennenberg, 2004).

Soybean is considered as an alternative crop to rice in rice-wheat cropping system in northwest India especially Punjab under crop diversification. However, the information on the effect of S use along with recommended N on yield, protein content and N:S ratio in soybean under Punjab agroclimatic conditions is lacking. Hence, the present study was aimed to determine the effect of N and S application on yield, N:S ratio and protein related parameters in developing soybean.

MATERIALS AND METHODS

A field experiment was conducted on soybean cv. 'SL 525' during *kharif* season 2012 in the experimental fields of Department of Plant Breeding & Genetics, PAU, Ludhiana (India). The experiment was laid out in a randomized block design with 4 treatments [control, nitrogen (urea @ 31.25 kg N ha^{-1}) and sulphur (gypsum @ 20 kg S ha^{-1}) alone and in combination]. Each treatment was replicated 4 times. The seeds were sown @ 75 kg ha^{-1}. Developing leaves, seeds, roots and stem were collected from 30 to 50 days after podding (DAP) at 10 days interval and dried at 60-70^{o}C. Dried leaves and seeds were powdered and used for the estimation of total soluble proteins (Lowry *et al.*, 1951), free amino acids (Lee and Takahashi, 1966) and S-containing amino acids *viz.*, methionine (Horn *et al.*, 1946) and cysteine (Gaitonde, 1967). Total N (Mckenzie and Wallace, 1954) and S (Chesnin and Yien, 1950) contents were determined in dried roots, stem, leaves and seeds. For N determination, dried samples were digested in presence of $CuSO_4$ + KSO_4 and H_2SO_4 at 400^{o}C in a digester and then distilled with 40% NaOH in Kelplus-Elite instrument. The titrations were done with a mixture of boric acid, few drops of bromocresol green and methyl red dye until end point was attained. For S estimation, dried samples were digested in a mixture of nitric: perchloric acid (5:2) and digested samples treated with 0.2 M sodium acetate buffer (pH 4.6), 0.25% gum acacia, solid $BaCl_2.2H_2O$. The absorbance was noted at 420 nm. Various yield parameters *viz.*, total seed yield, 100-seed fresh and dry weight were recorded at maturity. Means, standard deviation and critical differences for different parameters were assessed by applying factorial completely randomized design ($P \leq 0.05$, ANOVA) using CPCS1 software developed by PAU, Ludhiana (India).

RESULTS AND DISCUSSION

Total soluble proteins increased ~ 1.5- and 2.0-fold in leaves and developing seeds whereas free amino acids decreased significantly from 40 to 50 DAP in all the treatments (Table 1). Soluble proteins

Table 1: Effect of nitrogen and sulphur supply on total soluble proteins, free amino acids, methionine and cysteine contents in soybean leaves at different growth stages

Treatment*	Total soluble proteins (g 100 g^{-1} DW)***			Free amino acids (g 100 g^{-1} DW)			Methionine (mg g^{-1} DW)			Cysteine (mg g^{-1} DW)		
	30d**	40d	50d	30d	40d	50d	30d	40d	50d	30d	40d	50d
Control	2.19 ±0.06	1.78 ±0.07	2.62 ±0.10	4.21 ±0.07	3.81 ±0.27	2.76 ±0.43	1.95 ±0.31	1.25 ±0.15	0.19 ±0.02	1.20 ±0.21	1.38 ±0.17	0.82 ±0.05
N	2.40 ±0.17	2.25 ±0.09	3.79 ±0.24	5.25 ±0.60	4.10 ±0.69	3.42 ±0.35	2.15 ±0.29	1.35 ±0.22	0.36 ±0.02	1.36 ±0.15	1.44 ±0.26	0.84 ±0.11
S	2.64 ±0.21	2.23 ±0.10	3.39 ±0.13	6.23 ±0.35	4.82 ±0.38	4.28 ±0.75	3.13 ±0.19	1.45 ±0.04	0.59 ±0.09	1.41 ±0.06	1.62 ±0.09	0.96 ±0.17
N + S	2.65 ±0.36	1.97 ±0.24	3.16 ±0.29	5.47 ±0.31	3.99 ±0.21	2.95 ±0.43	3.81 ±0.23	1.33 ±0.32	1.12 ±0.08	1.23 ±0.14	1.58 ±0.21	0.88 ±0.01
$CD_{0.05}$ Fertilizer		0.16			0.37			0.16			ns	
Stage		0.14			0.32			0.14			0.11	

*N applied in the form of urea @ 31.25 kg ha^{-1} and S as gypsum @ 20 kg ha^{-1};
**d = Days after podding; *Data represent mean ± SD of four replications; ***DW = dry weight

Table 2: Effect of nitrogen and sulphur supply on nitrogen and sulphur content in leaves and seeds of soybean at different growth stages

Treat-ment*	Nitrogen (%) 30d**	Nitrogen (%) 40d	Nitrogen (%) 50d	Sulphur (%) 30d	Sulphur (%) 40d	Sulphur (%) 50d	N/S ratio 30d	N/S ratio 40d	N/S ratio 50d
Leaves									
Control	3.25±0.18	2.12±0.15	2.31±0.16	2.40±0.24	0.30±0.01	0.18±0.02	1.40±0.10	7.00±0.20	12.6±2.80
N	3.38±0.05	2.19±0.02	2.77±0.05	3.62±0.24	0.90±0.01	0.59±0.01	0.90±0.10	2.40±0.20	4.60±0.10
S	4.06±0.20	2.23±0.09	3.14±0.21	4.09±0.02	1.03±0.03	0.30±0.01	1.00±0.10	2.20±0.10	10.4±0.70
N + S	3.34±0.16	2.13±0.07	2.42±0.17	4.25±0.15	1.17±0.06	0.59±0.02	0.80±0.10	1.80±0.20	4.10±0.40
$CD_{0.05}$: Fertilizer	0.15				0.11			0.80	
Stage	0.13				0.09			0.70	
Stem									
Control	3.68±0.09	5.61±0.18	5.96±0.09	0.45±0.01	1.46±0.17	2.07±0.17	8.10±0.60	3.90±0.40	2.90±0.20
N	5.55±0.04	5.62±0.07	6.41±0.18	0.50±0.02	2.07±0.08	2.65±0.33	11.1±0.50	2.70±0.10	2.40±0.10
S	5.52±0.02	5.98±0.07	6.03±0.55	0.78±0.02	2.37±0.31	3.23±0.17	7.10±0.20	2.50±0.30	1.80±0.10
N + S	5.41±0.27	6.10±0.17	6.34±0.11	0.50±0.02	1.81±0.01	2.19±0.20	10.7±0.70	3.30±0.10	2.90±0.20
$CD_{0.05}$: Fertilizer	ns				0.17			1.60	
Stage	0.73				0.14			1.40	

in leaves and developing seeds increased significantly in N or S treatments alone or in combination as compared to control. Total soluble proteins increased significantly in developing seeds in all the treatments with maximum increase of 17.8% in N treatment at 40 DAP. The application of N and S alone or in combination significantly increased free amino acids in leaves as compared to control with maximum increase of 19.3 and 35.5% in N and S treatments, respectively, at 50 DAP. Methionine content in leaves was significantly higher in all the treatments than control from 30-50 DAP, except with N application alone or in combination with S at 40 DAP.

Nitrogen content in leaves increased at 50 DAP by 16.6 and 26.4% as a result of N or S application, respectively (Table 2). Sulphur in leaves decreased from 30 to 50 DAP whereas N:S ratio showed a reverse trend. A significant increase in S content in all the treatments as compared to control decreased N:S ratio with higher decrease of 3-4 folds in combined N and S treatment.

N content in roots and stems tended to decline as tissue aged but increased significantly in seeds in all the treatments (Tables 2 & 3). N application significantly increased N content in roots as compared to control from 30 to 50 DAP but only at 30 DAP by S. Sulphur treatment increased N content in stem at 30 and 40 DAP by 16.5 and 22.2%, respectively. S content of seeds increased by

Table 3: Effect of nitrogen and sulphur supply on nitrogen and sulphur content in soybean roots and stems at different growth stages

Treat-ment*	N content (%) 30d**	N content (%) 40d	N content (%) 50d	S content (%) 30d	S content (%) 40d	S content (%) 50d	N/S ratio 30d	N/S ratio 40d	N/S ratio 50d
Leaves									
Control	0.92±0.01	0.74±0.03	0.68±0.04	0.32±0.02	1.33±0.06	0.61±0.01	2.80±0.20	0.60±0.10	1.10±0.10
N	1.33±0.12	0.83±0.04	0.75±0.03	0.54±0.07	1.59±0.20	1.11±0.08	2.50±0.10	0.50±0.10	0.70±0.10
S	1.14±0.13	0.75±0.02	0.71±0.01	0.82±0.08	1.82±0.10	1.26±0.04	1.40±0.20	0.40±0.10	0.60±0.10
N + S	0.98±0.01	0.82±0.01	0.72±0.01	0.58±0.04	1.47±0.14	1.11±0.02	1.60±0.10	0.50±0.10	0.70±0.10
$CD_{0.05}$ Fertilizer	0.15				0.11			0.80	
Stage	0.13				0.09			0.70	
Stem									
Control	0.86±0.08	0.35±0.01	0.28±0.01	1.67±0.15	1.35±0.15	0.90±0.01	0.52±0.10	0.26±0.10	0.31±0.10
N	0.82±0.06	0.37±0.01	0.32±0.01	1.71±0.16	1.43±0.04	1.19±0.01	0.48±0.10	0.25±0.10	0.27±0.10
S	1.03±0.08	0.45±0.03	0.31±0.01	2.30±0.16	1.66±0.15	1.40±0.16	0.44±0.10	0.27±0.10	0.22±0.10
N + S	0.77±0.02	0.39±0.01	0.32±0.01	1.98±0.15	1.82±0.01	1.62±0.03	0.39±0.10	0.22±0.01	0.19±0.20
$CD_{0.05}$ Fertilizer	0.04				0.12			0.04	
Stage	0.03				0.10			0.03	

*days after poddling

Table 4: Effect of nitrogen and sulphur supply on total seed yield, 100 seed fresh and dry weight of soybean seeds at maturity

Treatment	Total seed yield (kg plot^{-1})	100-seed fresh weight (g)	100-seed dry weight (g)
Control	1.32 ± 0.02	9.28 ± 0.06	8.54 ± 0.05
N (urea @ 31.25 kg ha^{-1})	1.39 ± 0.03	9.62 ± 0.10	9.03 ± 0.10
S (gypsum @ 20 kg ha^{-1})	1.46 ± 0.03	10.08 ± 0.08	9.29 ± 0.06
N + S	1.56 ± 0.08	10.43 ± 0.11	9.33 ± 0.12
$CD_{0.05}$	0.09	0.18	0.17

*Data represent mean ± SD of four replications

~ 1.3- and 1.6-fold in N and S treatments, respectively, thus lowering the N:S ratio in these treatments. Total seed yield plot^{-1} and fresh and dry weight of 100-seeds increased by nitrogen, sulphur and their combined treatment as compared to control and dry weight/seed increased maximally from 85 mg to 93 mg in combined treatment of nitrogen and sulphur (Table 4). The increase in protein content by nitrogen or sulphur has previously been reported (Eman, 2002; Morshed *et al.*, 2008). Nitrogen concentration was higher in leaves followed by roots and stems at all the developmental stages. Low nitrogen concentration in stems than roots suggested that stems were capable of supplying limited N needs of developing seeds and probably roots were directly involved in N redistribution caused by rapid development of soybean (Fazili *et al.*, 2008). During pod filling stage, the increase in N concentration in the seeds and decrease in other tissues in all the treatments could be due to the fact that plants were still at last reproductive growth stages as there was seed production on lateral branches and these seeds were still accumulating dry matter and N content. In legumes, about 70% N is partitioned to leaf before commencement of pod growth (Muchow *et al.*, 1993) and it declines at or near the beginning of pod filling (Pengelly *et al.*, 1999). A large portion of leaf and stem N was remobilized to the grain. The decline in stem N before the beginning of seed growth was a consequence of nitrogen dilution and its decline after beginning of seed growth was due to remobilization of stem N to the developing grains (Soltani *et al.*, 2006). Most of the nitrogen used for grain filling was derived from mobilization of nitrogen stored in vegetative tissues (Rosato *et al.*, 2001) and active N_2 fixation at pod filling stage was more important to grain yield of soybean crop (Egli *et al.*, 1978). Leaves have been demonstrated as the main source of redistributed N. The concomitant occurrence of decrease in leaf N and increase in seed N during pod-filling stage suggested the dependence of soybean seeds on leaves for nitrogen supply.

Sulphur plays important role in formation of S-containing amino acids *viz.*, methionine and cysteine, synthesis of protein and oil in seeds (Jamal *et al.*, 2005, 2009). Sulphur is required to achieve maximum efficiency of applied N fertilizer (Fazili *et al.*, 2008). In present study, soybean leaf S content N:S ratio and N:S ratio decreased during seed-fill period. Sulphur fertilization affected nitrogen assimilation as indicated by enhanced protein accumulation rather than soluble N accumulation in seed. Since N:S ratio is an indicator of legume quality (Eppendorfer, 1971), decrease in this ratio suggested more S uptake. A decrease in N:S ratio with S and N treatments in mungbean seeds has previously been reported (Kumar *et al.*, 2013). Similarly, decreased N:S ratio with increasing dose of sulphate fertilizers in cowpea have been reported (Evans *et al.*, 2006). Najar *et al.* (2011) reported minimum N:S ratio with use of 40 kg S ha^{-1} over control treatment in soybean seeds. On contrary, N:S ratio increased in soybean under S stress (Sexton *et al.*, 1998). Sharma and Gupta (1992) reported that the application of 60 kg S ha^{-1} caused significant increase in sulphur and nitrogen content, whereas treatment with 40 kg S ha^{-1} increased sulphur content in mustard seeds (Fazili *et al.*, 2010).

Acknowledgement: The authors are thankful to Dr. Parampal Singh Gill for providing digestion (Kel Plus-KES42) and distillation apparatus (Kel Plus-ELITE, Pelican) for nitrogen estimation.

REFERENCES

Bellaloui, N., and Gillen, A.M. 2010. Soybean seed protein, oil, fatty acids, N, and S partitioning as affected by node position and cultivar differences. *Agricultural Sciences,* **1**: 110-118.

Chesnin, L. and Yien, C.H. 1950. Turbidtmetric determination of available sulphates. *Soil Science Society of America Proceedings,* **15**: 149-151.

Duraisami, V.P. and Mani, A.K. 2001. Residual effect of inorganic nitrogen, composted coirpith and biofertilizer on yield and uptake of soybean in an Incepstisol. *Madras Agricultural Journal,* **88**: 277-280.

Egli, D.B., Leggett, J.E. and Duncan, W.G. 1978. Influence of N stress and N redistribution in soybean. *Agronomy Journal,* **70**: 43-47.

Eman, S.S. 2002. Response of growth, yield and attributes of soybean plants [*Glycine max* (L.) Merr.] to late soil nitrogen fertilization. *Arab Universities Journal of Agricultural Sciences,* **10**: 165-172.

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. *Journal of the Science of Food and Agriculture,* **22**: 501-505.

Evans, I.M., Boulter, D., Robert, L., Fox, B. and Kang, T. 2006. The effects of sulphur fertilizers on the content of sulpho-amino acids in seeds of cowpea (*Vigna unguiculata*). *Journal of the Science of Food and Agriculture,* **28**: 161-166.

Fazili, I.S., Masoodi, M., Ahmad, S., Jamal, A., Khan, J.S. and Abdin, M.Z. 2010. Oil biosynthesis and its related variables in developing seeds of mustard (*Brassica juncea* L.) as influenced by sulphur fertilization. *Journal of Crop Science and Biotechnology,* **13**: 39-46.

Fazili I.S., Jamal, A., Ahmad, S., Masoodi, M., Khan, J.S. and Abdin, M.Z. 2008. Interactive effect of sulphur and nitrogen on nitrogen accumulation and harvest in oilseed crops differing in nitrogen assimilation potential. *Journal of Plant Nutrition,* **31**: 1203-1220.

Gaitnode, M.K. 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochemical Journal,* **104**: 627-633.

Horn, M.J., Jones, B. and Blum, A.E. 1946. Colorimetric determination of methionine in proteins and foods. *Journal of Biological Chemistry,* **166**: 313-320.

Jamal, A., Fazili, I.S., Ahmad, S., Abdin, M.Z. and Yun, S.J. 2005. Effect of sulphur and nitrogen application on growth characteristics, seed and oil yield of soybean cultivars. *Korean Journal of Crop Science,* **50**: 340-345.

Jamal, A., Ko, K., Kim, H.S., Cho, Y.K., Joung, H. and Ko, K. 2009. Role of genetic factors and environmental conditions in recombinant protein production for plant molecular biofarming. *Biotechnology Advances,* **27**: 914-923.

Kopriva, S. and Rennenberg, H. 2004. Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. *Journal of Experimental Botany,* **55**: 1831-1842.

Kumar, A., Sharma, S., Sital, J.S. and Singh, S. 2013. Effect of sulphur and nitrogen nutrition on storage protein quality in mungbean [*Vigna radiata* (L.) Wilczek] seeds. *Indian Journal of Agricultural Biochemistry,* **26**: 86-91.

Kumawat, S.M., Dhakar, L.L. and Maliwal, P.L. 2000. Effect of irrigation regimes and nitrogen on yield, oil content and nutrient uptake of soybean (*Glycine max*). *Indian Journal of Agronomy,* **45**: 361-366.

Lee, Y.P. and Takahashi, T. 1966. An improved colorimetric determination of amino acids with use of ninhydrin. *Analytical Biochemistry,* **14**: 71-77.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry,* **198**: 265-275.

Mckenzie, H.A. and Wallace, H.S. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions – temperature, catalyst and oxidizing agent. *Australian Journal of*

Chemistry, **7**: 3-39.

Morshed, R.M., Rahman, M.M. and Rahman, M.A. 2008. Effect of nitrogen on seed yield, protein content and nutrient uptake of soybean (*Glycine max* L.). *Journal of Agricultural and Rural Development,* **6**: 13-17.

Muchow, R.C., Robertson, M.J. and Pengelly, B.C. 1993. Accumulation and partitioning of biomass and nitrogen by soybean, mungbean and cowpea under contrasting environmental conditions. *Field Crops Research,* **33**:13-36.

Najar, G.R., Singh, S.R., Akhtar, F. and Hakeem, S.A. 2011. Influence of sulphur levels on yield, uptake and quality of soybean (*Glycine max*) under temperate conditions of Kashmir valley. *Indian Journal of Agricultural Sciences,* **81**: 340-343.

Pengelly, B.C., Blamey, F.P.C. and Muchow, R.C. 1999. Radiation interception and the accumulation of biomass and nitrogen by soybean and three tropical annual forage legumes. *Field Crops Research,* **63**: 99-112.

Rosato, L., Laine, P. and Ourry, A. 2001. Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns. *Journal of Experimental Botany,* **52**: 1655-1663.

Sexton, P.J., Naeve, S.L., Paek, N.C. and Shibles, R. 1998. Sulphur availability, cotyledon nitrogen:sulphur ratio and relative abundance of seed storage proteins of soybean. *Crop Science,* **38**: 983-986.

Sharma, R.A. and Gupta, R.K. 1992. Response of rainfed soybean-safflower sequence to nitrogen and sulphur fertilization in vertisols. *Indian Journal of Agricultural Sciences,* **62**: 529-534.

Soltani, A., Hammer, G.L., Torabi, B., Robertson, M.J. and Zeinali, E. 2006. Modeling chickpea growth and development: Nitrogen accumulation and use. *Field Crops Research,* **99**: 24-34.

Vasilas, B.L., Nelson, R.L., Fuhrmann, J.J. and Evans, T.A. 1995. Relationship of nitrogen utilization patterns with soybean yield and seed-fill period. *Crop Science,* **35**: 809-813.