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Effect of nitrogen and sulphur nutrition on yield parameters and protein composition in soybean [*Glycine max* (L.) Merrill].

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Abstract Accumulation patterns of various protein fractions in seed, yield and protein quality parameters of soybean cultivar SL 525 harvested at different stages of development from plants grown under different treatments *viz.,* nitrogen (urea @ 31.25 kg N ha^{-1}), sulphur (gypsum @ 20 kg S ha^{-1}) and combined application of nitrogen (N) and sulphur (S) were investigated. Application of S alone or combined with N significantly increased plant height, number of seeds per pod and total seed yield. Sulphur-containing amino acids *viz.,* methionine and cysteine increased significantly ($P \leq 0.05$) by all the treatments in comparison to control and maximum increase of 1.3 fold was observed by urea application. The contents of different protein fractions *viz.,* albumin, globulin and glycinin (a subfraction of globulin) increased under the effect of N and S applied alone or in combination from 30 DAP to maturity with maximum accumulation at maturity as compared to control (without N or S application). Gypsum @ 20 kg S ha^{-1} resulted in higher accumulation of glycinin (11S) relative to β-conglycinin (7S) fraction of globulin protein in soybean seed thereby increasing 11S:7S ratio indicating improvement in soybean seed quality. The current study showed that improvement of soybean nutritional quality can be achieved by application of gypsum @ 20 kg S ha^{-1} along with recommended N doses under agroclimatic conditions of Punjab.

Keywords: Nitrogen, Protein quality, Soybean, Sulphur, Yield

INTRODUCTION

Legume seeds contain high amounts of storage proteins that are classified on the basis of their solubility as water - soluble albumins, salt - soluble globulins, alcohol - soluble prolamins and acid - or alkaline - soluble glutelins (Utsumi, 1992). These seed storage proteins are synthesized in cotyledons during mid to later stages of crop development (Golombek *et al.*, 2001). Soybean is an important commercial leguminous crop grown throughout the world and is major source of protein, oil, fiber, vitamins, minerals and other nutrients. Its seed contains 40-45% protein on dry weight basis. The most abundant seed storage proteins of soybean are salt-soluble globulins and they consist of three fractions *viz.*, glycinin, β-conglycinin and 2S proteins. Glycinin accounts for 60% of storage proteins and is relatively rich in S-containing amino acids (3-4.5%) (Kaviani and Kharabian, 2008). It is a hexameric, 11S globulin (Mr 320-350 kDa), composed mainly of subunits A3; total A1a, A1b, A2, A4 and acidic and total basic with molecular weight 45, 38 and 22 kDa, respectively. β - conglycinin (7S) is a trimeric protein (Mr 180-240 kDa) and consists of α′, α and β subunits with molecular weight 76, 72 and 53 kDa, respectively (Remkema *et al.*, 2001). Subunits of β - conglycinin contain no more than 1.1% of S-containing amino acids (Krishnan *et al*., 2005) and β -subunit of β-conglycinin is poor in quality and usually lacks limiting S-containing amino acids (Tierney *et al.*, 1987).

The seed protein composition depends upon various environmental factors such as temperature, photoperiod and nutrition etc during seed development (Arslanoglu *et al.*, 2011; Kumar *et al.*, 2013). Alterations in the nutritional conditions may cause a wide range of morphological, physiological and biochemical changes by modification of genes and enzymes expression and thus protein synthesis and activity (Fabre and Planchon, 2000). Protein synthesis in soybean is reported to be highly influenced by minerals such as phosphorus, potassium, nitrogen and sulphur (Peak *et al.*, 1997; Utsumi, *et al.*, 2002; Mahmoodi *et al.*, 2013). Soybean plants grown under varying levels of sodium and potassium salts influenced seed storage protein subunit composition (Kaviani *et al.*, 2011). Sulphur as well as N are important nutrients required by crops for protein structure, vitamins and other structural components and also improve plant growth and yield (Marshner, 2005; Kopriva *et al.*, 2002).

Nitrogen and S assimilation is well coordinated and deficiencies of one of the nutrient reduce the assimilation of other nutrient (Kopriva and Rennenberg, 2004;

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Siddiqui *et al.*, 2008). Also, an insufficient supply of S can affect yield and quality of crop adversely (Scherer, 2001).

Soybean is considered as an alternative to rice in rice-wheat cropping system in Punjab due to its high nutritional quality and low water requirement. In a pot experiment, S as gypsum @ 20 kg ha^{-1} along with urea @ 31.25 Kg N ha^{-1} significantly increased nutrient uptake and seed yield (Sharma *et al.*, 2014). In our earlier work, we have studied the effect of various sources and levels of S fertilizers on storage protein accumulation in soybean crop to find out the optimum levels of S under the agroclimatic conditions of Punjab and observed that gypsum @ 20 kg S ha^{-1} showed maximum seed storage proteins accumulation in soybean (Kaur, 2014). In the present investigation, gypsum @ 20 Kg S ha^{-1} along with recommended dose of urea @ 31.25 Kg N ha^{-1} has been studied to evaluate the interactive effects of N and S application on protein quality and soybean yield.

MATERIALS AND METHODS

Study area: A field experiment was conducted at experimental fields of Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana during *kharif* season (2013) on soybean cultivar SL 525. The experiment was laid out with four replications in a randomized block design with four treatments i.e. control, urea @ 31.25 kg N ha^{-1}, gypsum @ 20 kg S ha^{-1} and urea @ 31.25 kg N ha^{-1} + gypsum @ 20 kg S ha^{-1}. The experimental soil was sandy loam in texture having pH 7.7, electrical conductivity 0.15 mmoles cm^{-1}, organic carbon 0.51 % and available nitrogen, phosphorus, potassium and sulphur contents were 0.26 %, 14.3 kg $acre^{-1}$, 30 kg $acre^{-1}$ and 0.22 % respectively. Seeds were collected at 30, 40 and 50 days after podding (DAP) and maturity from each treatment. Developing and mature seeds were defatted with chloroform and methanol in 2:1 ratio by overnight extraction at room temperature. After centrifugation, supernatant was discarded and defatted seed flour was used for storage protein extraction and analysis.

Different protein fractions were extracted from defatted soybean seed flour based on their solubility at 25^oC in distilled water (for albumin), 0.2 M sodium phosphate buffer pH 8.0 containing 3% NaCl (for globulin), 70% ethanol (for prolamin) and 0.1 N NaOH (for glutelin) following the procedure given by Pant and Tulsiani (1969). The subfractions of globulins *viz.*, glycinins and β-conglycinins were extracted from defatted soybean flour with 5-6 volumes of 0.1M potassium phosphate buffer (pH 7.0) and further, isolated by repeated isoelectric precipitations (Basha and Beevers, 1975) where glycinins were recovered at pH 6.4 while β-conglycinins were obtained at pH 4.8. The protein content of various fractions was determined by the method of Lowry *et al.* (1951). Mature seeds were also analyzed for total soluble proteins (Lowry *et al.*, 1951), free amino acids (Lee and Takahashi, 1966), S-containing amino acids *viz.*, methionine (Horn *et al.*, 1946) and cysteine (Gaitonde, 1967). Various yield parameters *viz.*, seed yield, seeds per pod, pods per plant and plant height were determined at maturity before harvesting.

Statistical analysis: The data was analysed for the critical differences between treatments and developmental stages by applying one way analysis of variance (ANOVA) at 5% level of significance using CPCS1 software package, developed by PAU, Ludhiana.

RESULTS AND DISCUSSION

The accumulations of albumin and globulin proteins increased significantly from 30 DAP to maturity in all the treatments (Table 1). Albumin content in seeds increased significantly ($P \leq 0.05$) in response to all the treatments as compared to control at various developmental stages and maximum increase was observed by application of urea alone @ 31.25 Kg N ha^{-1} at each stage of development. Accumulation of globulins showed significant ($P \leq 0.05$) increase in all the treatments and their content varied significantly among N, S and their combined application. The maximum globulin accumulation in mature seeds was observed by S application.

Prolamin content decreased significantly from 30 to 50 DAP and increased thereafter up to maturity in all the treatments (Table 1). Maximum accumulation of prolamins was observed by the application of gypsum alone from 30 to 50 DAP while at maturity, the maximum response was given by combined application of N and S. The accumulation of glutelins decreased significantly in mature seeds compared to the initial stages of development in all the treatments. Various treatments significantly increased the seed glutelin content compared to control at maturity. Prolamin and glutelin accumulation was highest in the combined application of N and S in mature seeds.

The glycinin (11S) fractions of globulin storage protein increased significantly from 30 DAP to maturity (Fig. 1) in all the treatments. Glycinin content increased by the application of N and S alone or in combination compared to control and maximum accumulation of glycinin fraction was observed at maturity in each treatment. The accumulation of β-conglycinin (7S) increased from 30 to 40 DAP in all the treatments and then decreased further up to 50 DAP (Fig. 1). The relative proportion of β-conglycinin decreased significantly ($P \leq 0.05$) by the application of S alone at all the developmental stages and maturity. N treatment alone or in combination with S resulted in increased accumulation of β-conglycinin in mature seeds. 11S:7S ratio initially decreased upto 40 DAP and then increased up to maturity in all the treatments and maximum increase was observed in S treatment.

Total soluble proteins, free amino acids content

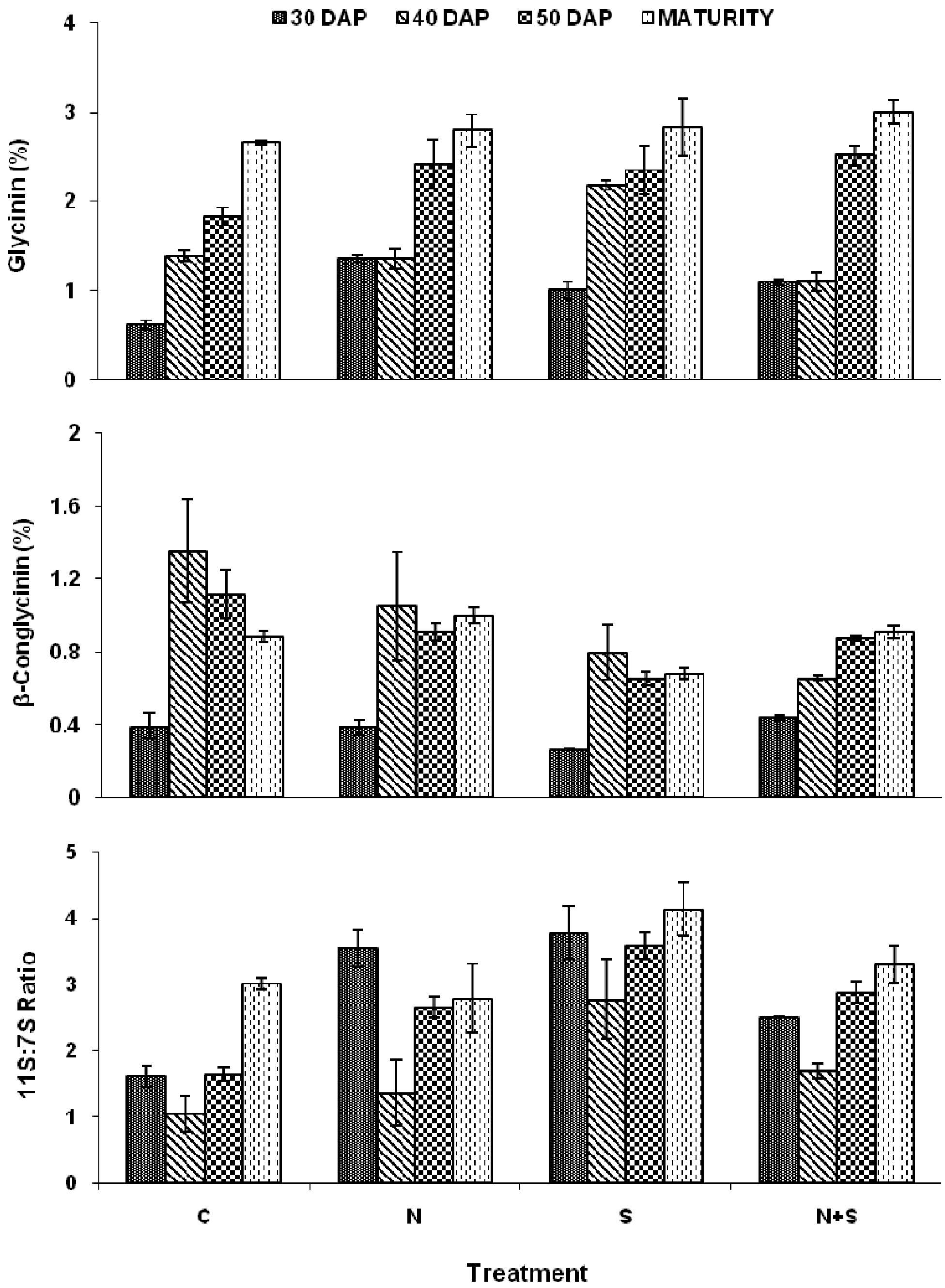

Fig. 1. *Effect of nitrogen and sulphur fertilization on glycinin (11S), β-conglycinin (7S) content and 11S:7S ratio in soybean seeds at different developmental stages. (C: control; N: urea @ 31.25 kg ha^{-1}; S: gypsum @ 20 kg ha^{-1}; N+S: urea @ 31.25 kg ha^{-1} + gypsum@ 20 kg ha^{-1}).*

increased after treatment with N or S alone or in combination but the contents varied non-significantly ($P \leq 0.05$). Methionine and cysteine content in seeds increased significantly in all the treatments in comparison to control (Table 2).

In addition to protein quality, various yield parameters determined at maturity were also affected by all fertilizer treatments (Table 3). Plant height and number of seeds per pod increased significantly by the application of N or S alone and in combination as compared to control. Seed yield increased significantly by gypsum alone @ 20 Kg S ha^{-1} or by its combination with N.

In present studies, the major proteins in soybean seeds were globulins, followed by albumins, glutelin and prolamins being the lowest. Globulins, being the major seed storage proteins in legumes, had the highest relative content in mature soybean seeds and are synthesized during seed development on polysomes, transferred via lumen, sequestered and ultimately deposited in protein bodies (Kaviani and Kharabian, 2008). Increase in albumin and globulin content in chickpea seeds by various S sources including single super phosphate,

Table 1. Effect of nitrogen and sulphur fertilization on storage protein composition of soybean seeds at different developmental stages.

Treatment	Albumins (%)				Globulins (%)			
	30 DAP	40 DAP	50 DAP	Maturity	30 DAP	40 DAP	50 DAP	Maturity
Control	2.32±0.02	2.60±0.05	2.90±0.01	11.30±0.20	3.19±0.01	8.83±0.25	6.60±0.05	20.49±0.21
Nitrogen @ 31.25 kg ha^{-1} urea	3.20±0.10	3.35±0.10	3.45±0.15	12.80±0.20	6.40±0.12	9.83±0.41	7.53±0.22	22.31±0.03
Sulphur @ 20 kg ha^{-1} gypsum	2.93±0.05	3.05±0.05	3.40±0.10	11.95±0.05	5.34±0.16	9.96±0.36	6.63±0.15	22.65±0.17
Nitrogen @ 31.25 kg ha^{-1} urea + Sulphur @ 20 kg ha^{-1} gypsum	2.80±0.10	2.85±0.01	3.25±0.15	12.05±0.05	3.40±0.09	8.94±0.18	5.44±0.36	21.96±0.31
Critical Difference (P≤0.05)								
Treatment	0.09				0.19			
Stage	0.09				0.19			
	Prolamins (%)				Glutelins (%)			
	30 DAP	40 DAP	50 DAP	Maturity	30 DAP	40 DAP	50 DAP	Maturity
Control	1.30±0.05	1.13±0.02	0.33±0.03	0.41±0.01	5.67±0.07	7.45±0.10	2.75±0.01	2.76±0.05
Nitrogen @ 31.25 kg ha^{-1} urea	1.35±0.05	1.18±0.02	0.25±0.01	0.58±0.01	5.90±0.05	5.39±0.07	2.25±0.01	3.17±0.06
Sulphur @ 20 kg ha^{-1} gypsum	1.58±0.02	1.38±0.02	0.50±0.01	0.58±0.03	5.72±0.02	5.97±0.08	2.51±0.03	2.83±0.03
Nitrogen @ 31.25 kg ha^{-1} urea + Sulphur @ 20 kg ha^{-1} gypsum	1.13±0.02	0.80±0.05	0.10±0.01	0.67±0.01	5.93±0.12	6.90±0.01	2.58±0.08	3.42±0.05
Critical Difference (P≤0.05)								
Treatment	0.02				0.05			
Stage	0.02				0.05			

Values are mean ± SD of four replicates ; DAP : days after podding

Table 2. Effect of nitrogen and sulphur fertilization on total soluble proteins, amino acids, methionine and cysteine content of mature soybean seeds.

Treatment	Total soluble proteins (%)	Free amino acids (%)	Methionine (%)	Cysteine (%)
Control	21.25 ± 0.37	1.58 ± 0.14	0.57 ± 0.04	0.22 ± 0.01
Nitrogen @ 31.25 kg ha-1 urea	22.13 ± 0.81	1.92 ± 0.07	0.75 ± 0.05	0.30 ± 0.01
Sulphur @ 20 kg ha-1 gypsum	22.66 ± 0.65	1.88 ± 0.25	0.73 ± 0.09	0.27 ± 0.01
Nitrogen @ 31.25 kg ha-1 urea + Sulphur @ 20 kg ha-1 gypsum	22.88 ± 0.81	1.92 ± 0.01	0.70 ± 0.05	0.26 ± 0.01
Critical Difference (P≤0.05)	NS	NS	0.12	0.02

Values are mean ± SD of four replicates, NS: Non-significant.

Table 3. Effect of nitrogen and sulphur fertilization on yield parameters of soybean**.**

Treatment	Seed Yield (kg/plot)	Pods/plant	Seeds/pod	Plant Height (cm)
Control	1.27 ± 0.14	103.33 ± 5.03	2.05 ± 0.09	67.66 ± 2.51
Nitrogen @ 31.25 kg ha-1 urea	1.30 ± 0.08	82.67 ± 4.50	2.15 ± 0.12	71.67 ± 0.57
Sulphur @ 20 kg ha-1 gypsum	1.65 ± 0.10	89.00 ± 8.00	2.50 ± 0.06	73.67 ± 1.52
Nitrogen @ 31.25 kg ha-1 urea + Sulphur @ 20 kg ha-1 gypsum	1.62 ± 0.07	106.33 ± 14.46	2.23 ± 0.07	75.33 ± 1.15
Critical Difference (P≤0.05)	0.20	16.82	0.16	3.03

Values are mean ± SD of four replicates.

gypsum and elemental S at all developmental stages (Ghalotra *et al.*, 2007), by N and S in chickpea (Singh and Matta, 2005) and mungbean (Kumar *et al.*, 2013) have been reported whereas seed globulin decreased in lupin seeds due to S deficiency (Gillespie *et al.*, 1978). In the present studies, the increase in globulin and albumin contents in soybean seeds with N and S supply alone or in combination suggested that supply of various minerals can improve the storage protein fractions in soybean.

There was higher accumulation of 11S fractions at maturity with N and S treatments as glycinin proteins are reported to reach maximum transcription level at 70-80 DAF (Nielsen *et al.*, 1989). There was decreased accumulation of β-conglycinin due to N and S alone or in combination except that N application increased its content at maturity. Peak *et al.* (1997) have shown that nitrogen source has a profound effect on the relative amounts of β-subunit of β-conglycinin and that the accumulation of β-subunit is controlled by N availability. Eliminating or reducing the β-subunit has been suggested as one method of improving the quality of soybean proteins (Wilson, 1987). Accumulation of S-poor β-subunit of β-conglycinin was promoted by excess application of N or by S deficiency while the application of S fertilizers increased glycinin synthesis (Krishnan, *et al.*,, 2000; Awazuhara *et al.*,, 2002 and Krishnan, *et al.*, 2005). The S-poor β-subunit of β-conglycinin is more strongly expressed under nitrogen nutritional conditions that promote 7S protein, whereas S-containing subunits are influenced less or not at all, thereby deteriorating the 11S/7S ratio. β-subunit of β-conglycinin demonstrated plasticity in response to S availability (Tabe *et al.*,, 2002; Hagan *et al.*, 2003) which modulated the expression of transcripts and genetic developmental program (Rolletschek *et al.*, 2005).

Availability of S-amino acids in the developing seeds determined which storage protein subunits were synthesized from the extra N. The increase in contents of S-containing amino acids by N and S alone or in combination in present studies suggest improvement of seed storage protein quality. Sulphur fertilization increased cysteine and glutathione content of leaves while N fertilization had no significant influence (Bloem *et al.*, 2010) at all the developmental stages in garlic.

In the present studies, plant height, seeds per pod and seed yield improved by S alone or combined with N whereas N alone decreased number of pods per plant thus showing non-significant (P≤0.05) variation in seed yield as compared to control. Sulphur application @ 40 kg ha^{-1} enhanced the plant height, branches, pod/ plant and 1000g weight in green gram (Sharma and Singh, 1997), black gram (Singh and Aggarwal, 1998) and soybean (Ganeshamurthy and Reddy, 2000). The improved growth and physiological attributes appear to be mainly responsible for the observed parallel increase in the various yield characteristics studied. Nitrogen and S assimilation was enhanced due to fertilizer treatment that can be correlated to yield.

Conclusion

From the present studies, it was concluded that gypsum @ 20 kg S ha^{-1} alone or in combination with recommended doses of nitrogen for soybean improved the total storage protein, glycinin fraction of globulin, S-containing amino acids and seed yield in soybean under agro-climatic conditions of Punjab whereas the content of S-deficient β-conglycinin fraction decreased thus improving 11S:7S ratio indicating improvement in protein quality of soybean. Hence, S as gypsum @ 20 kg ha^{-1} along with urea @ 31.25 kg N ha^{-1} can be used to improve yield and storage protein quality of soybean under local climatic conditions.

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