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Effect of nitrogen and sulfur applications on growth, chlorophyll content and yield of soybean [*Glycine max* (L.) Merr.]

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ABSTRACT

The current investigation reports the effect of basal and splitting application of nitrogen and sulfur on growth, chlorophyll content and yield at R_2 and R_5 growth stages of soybean in *kharif season* during year 2018. A field experiment was conducted at the Research Farm, Indian Institute of Soybean Research, Indore, India under different 14 treatment combinations involving different doses of N (12.5, 25 and 50 kg ha^{-1}) and S (12.5, 25 and 50 kg ha^{-1}) as basal and split application at R_2 growth stage of crop. The experiment was laid out in randomized block design with three replications. The results revealed that the basal and split application of nitrogen and sulfur induces the improvement in plant height, dry matter accumulation, chlorophyll content, leaf area, no. of branches per plant, pod per plant, seed index and grain yield over control. Average highest plant height, dry matter accumulation, chlorophyll content, leaf area, no. of branches per plant, pod per plant, seed index and grains yield were recorded with the application of N25+25, S25+25 at R_2 and R_5 growth stages. Thus, it can be concluded that split application N and S (N25+25, S25+25) can be applied to achieve better growth and yield in soybean crop.

Key words: Chlorophyll content, growth, nitrogen, soybean, sulfur, yield

INTRODUCTION

Soybean is one of the most important oil seed crops of India and the world. Majority of soybean area in India is in central part and is grown on vertisols and associated soils and by and large the crop is grown under rain fed situations. There is a considerable potential to bride the yield gap between the real and potential yield by adopting of appropriate improved resource management approaches. Nitrogen and sulfur are essential nutrients as they are involved in many biochemical processes and their deficiency not only affects crop productivity but also the quality of produce for human and animal consumption (Salvagiotti et al., 2008; Arata et al., 2017; Krishnan and Jez, 2018). Soybean has a large nutrient requirement throughout the growing season and has an especially high N requirement due to its seed protein content that averages about 40% based on seed dry weight (Bellaloui et al., 2015). Crop growth, grain yield will be increased when N fertilizer is added (Aulakh and Malhi, 2004). Nitrogen is a crucial plant nutrient, as it is an important component of chlorophyll. Nitrogen application time may be an important factor when considering N fertilizer application to soybean. Applying N at later stages may increase yield by supplying N at a vital time when N supply may be limited. Barker and Sawyer (2005) concluded that fertilizing soybean with N at the beginning of R3 increased yield over the control. Kinugasa et al. (2012) also reported that N fertilization after flowering significantly increased soybean seed number per plant. Over the last two decades, sulfur (S) deficiency has been recognized as a constraint on crop production all over the world

(Schonhof et al., 2007; Mascagni et al., 2008). The levels of the sulfur-containing amino acids cysteine and methionine in soybean seed proteins are inadequate for optimal growth and development of monogastric animals and the role of sulfur in plant metabolism, human and animal diet assumes significance (Krishnan and Jez, 2018). Fertilization with S enhances the effect of N and intervenes in soil processes that improve N-use efficiency by the crop. This improvement has been shown to be due to greater N recovery, without changes in internal efficiency (Salvagiotti et al., 2008). Plants must be supplied adequately with nutrients during the critical growth period for the normal maintenance of their physiological and biochemical processes. For this reason, obviously the concentration of plant nutrients in the soil solution must be maintained at a satisfactorily level for optimum plant growth. Therefore, the present study was undertaken to develop an optimum N and S management approach for soybean.

MATERIALS AND METHODS

Field experiment

A field experiment was conducted at the Research Farm, Indian Institute of Soybean Research, Indore, India (22° 8' N latitude; 75° 4' E longitude) during *kharif* 2018 (rainy season) to evaluate the effect of N and S application on growth and yield of soybean. The experimental soil (vertisols) belonged to Sarol soil series (Fine, iso-hyperthermic, montmorillonitic, Typic Haplusterts) with soybean-wheat as the predominant cropping pattern. The characteristics of the soil at zero time are as follows: pH 8.2, Organic carbon 4.6 g kg^{-1}, clay content 56.2%. The experiment consisted of 14 treatments involving different doses of N (12.5, 25 and 50 kg ha^{-1}) and S (25 and 50 kg ha^{-1}). Nitrogen and sulphur were applied as basal and split application at R_2 stage of soybean in various combinations. The experiment was laid out in randomized block design with three replications. The size of each plot was 6 m long and 3.6 m wide. All other recommended agronomic practices were followed to harmonize with prevalent practices. At R_2 and R_5 growth stage of soybean growth stage, three replicates were utilized for growth and yield parameters. Plant samples (5 plants) were collected randomly to assess plant height, branches per plant, pods per plant, dry matter accumulation. For dry matter accumulation plant samples were oven dried at 70° C till constant weight. Leaves from each plant were taken and fed into the automatic leaf area meter (CI-203, portable leaf area analyzer (CID, USA) to measure leaf area. The total chlorophyll content was determined by dimethyl sulfoxide (DMSO) method (Hiscox and Israelstam, 1979). For the extraction of chlorophyll 50 mg well-cleaned fresh leaf was chopped and transferred to a test tube containing 10 ml of DMSO. The contents were incubated at 65°C for 3 h and volume was made up to 10 ml with DMSO. The contents were allowed to settle down and the absorbance of supernatant was recorded at 645 and 663 nm. Finally, the total chlorophyll content was calculated by using the following formula:

Total Chlorophyll (mg g^{-1} leaf fresh weight) = [20.2 x (A645 + 8.02 + A663) V]/(1000 x W)

Where, A, V and W were absorbance, final volume, and weight of sample, respectively.

Statistical analysis

The data was analyzed by using SAS statistical software (ver.9.2; SAS Institute., Cary, NC). One-way analysis of variance (ANOVA) was carried out with the ANOVA procedure in SAS enterprise guide 4.2 and the Fisher least significant differences and Duncan multiple range test were used to separate the treatment means.

RESULTS AND DISCUSSION

Plant height

Data in Table 1 shows the effect of nitrogen and sulfur on plant height and dry matter accumulation. Plant height significantly increased with basal and split application of N and S as compared to control as well as alone and combined treatments of N and S at R_2 and R_5 growth stages of crop. The application of N25; N50 and split application of N and S significantly increased the plant height at R_2 and R_5 stages of crop growth. The highest plant height was recorded with N25+25, S25+25 which was statistically similar with N25; N50 at R_2 growth stage and significantly different from control and other treatment combination. At R5 stage the highest plant height recoded with N25+25, S12.5+12.5 which was at par with N25; N50; N25+25; N12.5+12.5, S25+25; N25+25, S25+25 and N25+S50 while the smallest plants were recorded in control in both stages. The almost same results were also reported by (Sharma et al., 2014; Khalili et al., 2021).

Table 1. Effect of different levels and methods of nitrogen and sulfur applications on dry matter and plant height at different stages of soybean crop growth

Treatment	Plant height (cm)		Dry matter accumulation (g $plant^{-1}$)	
	R_2	R_5	R_2	R_5
Control	46±3.51^{h}	66±9.29^{e}	8.80±0.40^{f}	17.39±2.18^{f}
N25	64±3.60^{abc}	81±3.60^{abc}	11.87±1.02^{abcd}	24.84±2.66^{abcd}
N50	65±3.51^{ab}	80±8.66^{abc}	12.93±0.83^{ab}	26.74±3.46^{abc}
N25+25	58±2.88^{cde}	83±2.64^{ab}	12.47±0.41^{ab}	27.36±2.47^{a}
N12.5+12.5	50±5.29^{gh}	72±2.51^{cde}	9.53±0.41^{f}	18.44±1.27^{ef}
S25	53±3.05^{efg}	74±5.68^{bcde}	9.47±2.53^{f}	19.72±1.55^{ef}
S50	54±4.58^{efg}	78±3.00^{abcd}	10.00±0.87^{def}	21.62±1.76^{de}
S12.5+12.5	46±1.52^{h}	69±3.60^{de}	8.80±0.20^{f}	17.83±1.13^{f}
S25+25	56±2.64^{def}	72±1.52^{cde}	10.33±2.46^{def}	23.62±1.18^{cd}
N25+25, S12.5+12.5	61±3.78^{bcd}	86±3.60^{a}	12.27±0.46^{abc}	28.02±1.26^{a}
N12.5+12.5, S12.5+12.5	51±3.05^{fgh}	72±9.00^{cde}	10.40±0.34^{cdef}	20.18±1.38^{ef}
N12.5+12.5, S25+25	57±4.16^{def}	79±2.88^{abc}	9.67±1.70^{ef}	23.93±1.83^{bcd}
N25+25, S25+25	69±3.00^{a}	82±9.60^{ab}	11.53±1.30^{bcde}	27.53±1.95^{a}
N25+S50	63±2.08^{bc}	77±4.58^{abcd}	13.73±0.41^{a}	27.11±2.75^{ab}
LSD (p=0.05)	10.35	17.13	3.41	6.08

Data are mean values of three replicates± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Dry matter accumulation

Alone N and combined with S fertilization significantly increased the dry matter accumulation of crop. At R_2 growth stage, significantly the highest dry matter accumulation was recorded with the application of N25+S50 which was not significantly different from N25; N50; N25+25 and significantly different from other treatments. At R_5 stage, the highest dry matter accumulation was produced by N25+25, S12.5+12.5 which was statistically same with N25+S50; N25; N50; N25+25 and N25+25, S25+25 and significantly different from other treatment combinations. At R_5 growth stage, combined split application of N and S (N25+25, S12.5+12.5; N25+25, S25+25) and alone split application N (N25+25) treatments were recorded significantly higher dry matter accumulation of crop over other treatments and control.

Chlorophyll content

There were no significant changes in chlorophyll content with the application of N and S at R_2 and R_5 stages of soybean, however, these treatments were significantly increased as compared to control (Table 2). The basal application of N25; N50; S50 and split application of N and S (N25+25, S25+25;

N25+25, S12.5+12.5; N25+S50) were recorded significantly higher chlorophyll content as compared to other treatments and control at R_2 growth stage. At R_5 growth stage, basal application of (N25; N50) and splitting of N and S (N25+25, S25+25; N25+S50; N25+25 with S12.5+12.5) recorded significantly higher chlorophyll content than other treatments and control. Leaf area surface was significantly increased by split application of N and S over control. Significantly higher chlorophyll content at different crop growth stages statement is endorsed by (Chavan et al., 2008; Sharma et al., 2022).

Table 2. Effect of different levels and methods of nitrogen and sulfur applications on total chlorophyll content and leaf area surface at different stages of soybean crop growth

Treatment	Total chlorophyll content (mg g^{-1} leaf fresh weight)		Leaf area surface (cm^2)	
	R_2	R_5	R_2	R_5
Control	28.99±2.33^{b}	45.43±4.74^{d}	955±174^{d}	1511±284^{e}
N25	45.07±9.63^{a}	53.63±3.43^{abcd}	1770±315^{abc}	2361±259^{abcd}
N50	43.26±4.38^{ab}	58.12±5.25^{ab}	1904±98^{ab}	2636±238^{ab}
N25+25	42.11±6.76^{ab}	55.38±2.00^{abcd}	1849±51^{ab}	2461±321^{abc}
N12.5+12.5	39.42±2.44^{ab}	49.57±1.86^{abcd}	1156±156^{cd}	2087±124^{abcde}
S25	39.76±6.48^{ab}	47.93±0.55^{cd}	977±42^{d}	1669±102^{ed}
S50	45.00±4.29^{a}	52.00±2.95^{abcd}	1107±114^{d}	1944±428^{bcde}
S12.5+12.5	36.81±2.30^{ab}	47.75±1.22^{cd}	942±218^{d}	1503±54^{e}
S25+25	40.79±0.82^{ab}	48.83±1.29^{bcd}	1186±69.47^{cd}	1977±295^{abcde}
N25+25, S12.5+12.5	44.16±3.85^{ab}	57.16±4.91^{abc}	2035±229^{a}	2400±23^{abc}
N12.5+12.5, S12.5+12.5	33.03±5.52^{ab}	46.53±5.01^{d}	1185±129^{cd}	1911±88^{cde}
N12.5+12.5, S25+25	38.92±2.32^{ab}	46.05±1.95^{d}	1286±299^{bcd}	1624±273^{e}
N25+25, S25+25	45.77±7.42^{a}	59.52±2.01^{a}	2190±505^{a}	2675±330^{a}
N25+S50	44.39±4.57^{a}	58.05±4.33^{ab}	2024±92^{a}	2444±196^{abc}
LSD (p=0.05)	15.19	10.01	647	715

Data are mean values of three replicates± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Leaf area

Application of (N25+25, S25+25) produced the maximum leaf surface area at R2 and R5 stages of crop over control which did not significantly various with basal application of N (N25; N50) and split application of N and S (N25+25; N12.5+12.5; N25+25, S12.5+12.5; N25+S50) and statistically differ from other treatments combinations together with control. These results are recommended by significantly higher leaf area at different stages of the crop by many scientists (Chavan et al., 2008; Sharma et al., 2022).

Branches per plant

The number of branches per plant was improved by alone nitrogen and combined with sulfur as basal and splitting fertilization (Table 3). The highest number of branches per plant were found with combined split application of N and S (N25+25, S25+25) which was statistically similar with (N50; N25+25; N25+25, S12.5+12.5; N12.5+12.5, S25+25; N25+S50) and various with other treatments.

Yield attributes

The data on no. of pods per plant revealed that highest pods yield was obtained with application of N25+S50, S25+25 which were at par with application of N25+S50 and N25+25 and it is significantly different from other treatments during the year of investigation (Table 3). These findings are in accordance with the results of Khalili et al. (2021). The seed index was significantly higher with the application of basal application of N50, and split application of N and S (N25+25; N25+25, S25+25; N25+25, S12.5+12.5; N25+S50) as compared to control and other fertilized treatment in the study and lowest seed index was found with the control. However, Khalili et al. (2021) reported that N rate and time of application did not influence the seed index of soybean.

Table 3. Effect of different levels and methods of nitrogen and sulfur applications on no. of branch per plant, pods per plant, seed index and grains yield at harvest

Treatment	No. of branch per plant	No. of pods per plant	Seed index (g)	Grains yield (kg ha^{-1})
Control	5±1.00^{d}	93±3.60^{g}	10.79±0.44^{g}	1918±57^{g}
N25	6±1.15^{bcd}	123±2.08^{bcde}	12.46±0.09^{abc}	2517±182^{bcde}
N50	7±1.00^{abc}	126±20.03^{bcd}	12.75±0.51^{a}	2724±345^{ab}
N25+25	7±0.57^{ab}	131±14.52^{abc}	12.34±0.99^{abcd}	2556±28^{bcd}
N12.5+12.5	7±0.00^{abc}	112±3.21^{def}	11.43±0.14^{efg}	2127±232^{fg}
S25	6±0.57^{cd}	109±16.28^{ef}	11.77±0.11^{bcdef}	2168±155^{efg}
S50	6±0.00^{bcd}	118±8.88^{bcdef}	11.87±0.68^{bcde}	2199±121^{defg}
S12.5+12.5	6±1.15^{cd}	92±6.42^{g}	11.55±0.29^{defg}	2056±102^{g}
S25+25	6±0.57^{bcd}	107±5.68^{efg}	11.91±0.91^{bcde}	2278±21^{def}
N25+25, S12.5+12.5	7±0.57^{ab}	127±8.73^{bcd}	12.54±0.34^{ab}	2684±302^{abc}
N12.5+12.5, S12.5+12.5	6±0.57^{cd}	104±3.05^{fg}	11.70±0.29^{cdef}	2156±157^{fg}
N12.5+12.5, S25+25	7±1.00^{abc}	117±7.54^{cdef}	11.02±0.18^{fg}	2347±182^{cdef}
N25+25, S25+25	8±0.57^{a}	145±4.35^{a}	12.43±0.45^{abc}	3011±397^{a}
N25+S50	7±1.15^{ab}	134±9.84^{ab}	12.20±0.26^{abcde}	2875±282^{ab}
LSD (p=0.05)	1.34	16.22	0.82	359

Data are mean values of three replicates ± SD; means with different letters in the same column differ significantly at P=0.05 according to Fisher LSD

Grain yield

Data presented in Table 3 indicated that the yield of grains in soybean was significantly affected by basal and split application of N and S alone and both by combined treatments. The highest seed yield was obtained with the application of N25+25, S25+25 and statistically similar with N25+25, S12.5+12.5; N25+S50; N50 and significantly different from other basal and splitting of N and S as well as control. However, the lowest grains yield was produced by the control. The above results are in conformity with those of Khalili et al. (2016), Mamatha et al. (2018) and Khalili et al. (2021) who reported that the application of N and S recorded significantly higher grain yield as compared to control in maize and soybean.

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