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Original ¹ Research Article

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3 Effect of phosphorus and sulphur on nutrient and amino acids content of soybean (*Glycine max*
4 L. Merrill) under 'Eutrochrepts'.

5 ABSTRACT

6 A field experiment was conducted at KVK, Srinagar during two consecutive *kharif* seasons of
7 2010 and 2011 to study the "Effect of phosphorus and sulphur on yield and quality of soybean
8 (*Glycine max* L. Merrill) under Eutrochrepts". The experiment was laid down under 16 treatment
combinations viz four levels of phosphorus (0, 30, 60, 90 kg P₂O₅ ha⁻¹) and four levels of sulphur (0,
15, 30, 45 kg S ha⁻¹) in randomized complete block design with three replication. At higher levels of
phosphorus application, Zn content of seed decreased and it was maximum at 30 kg P₂O₅ ha⁻¹. With
application of 45 kg S ha⁻¹, N, P, K, Ca, Mg and S content in seed was 6.54, 0.555, 1.881, 0.329,
13 0.434 and 0.501 per cent respectively while as Zn, Fe, Cu, Mn was 109.99, 99.96, 2.82 and 3.73 mg
kg⁻¹, respectively. A significant interaction between P and S on macro as well as micronutrient content
15 except Zn in seed was observed. Combined application of phosphorus and sulphur further enhanced
16 the nutrient content of soybean seed. Combined application of phosphorus and sulphur enhanced the
crude protein and oil content in soya seed 17. Individual as well as interaction effect of P and S was
18 significant in enhancing the sulphur containing amino acids viz., cystine cystein and methionine
content of soybean seed. Combined application of 45 kg S and 90 kg P₂O₅ ha⁻¹ 19 recorded significantly
20 higher carbohydrate content (23.49%) in soybean seed. Application of increasing levels of phosphorus
21 and sulphur resulted in gradual increase in linoleic (Omega-6) and linolenic acid (Omega-3).

22 **Key words:** Phosphorus, sulphur, soybean, Zn, Fe, Cu, Oil, fatty acids

23 INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] has become the miracle crop of the 21st 24 century. It
25 belongs to the family Leguminosae, sub-family Papilionaceae and the genus Glycine. It is a triple
26 beneficiary crop, which contains about 40 per cent protein, possessing high level of essential amino27
acids methionine and cystine, 20 per cent oil rich in poly unsaturated fatty acids especially omega-6
28 and omega-3 fatty acids, 6-7 per cent total minerals, 5-6 per cent crude fibre and 17-19 per cent
29 carbohydrates (Chauhan *et al.*, 1988). Besides, it has good amount of iron, vitamin B-complex and
30 isoflavones such as daidzein, genistein of glycitin. Presence of calcium and iron makes it highly
31 suitable for women who suffer from osteoporosis and anaemia. The isoflavones of soybean have been
32 found to possess health benefits, as they exhibited properties like cancer preventing, combating
33 menopausal problem and helping to recover from diabetes (Chauhan *et al.*, 2002).

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Soybean was considered only as a food and fodder crop till World War-II 34 when its potential 35 as an oilseed crop was realized. Due to its multifaceted uses, soybean has since progressed by leaps 36 and bounds as an oilseed crop. So much so that on the global scale it has come to the top of the list of 37 oilseed crops and contributes over one-third of the total supply of the world vegetable oil pool. 38 Indians as such, know soybean since ages as it was in cultivation in northern and north-eastern hills as 39 food plant and is a part of routine diet of the people (Tiwari *et al.*, 1999). Black-seeded soybean has 40 been grown since early times in the northern and north eastern hills and in scattered area in the central 41 part of the country. Soybean was introduced in India probably as soon as it was domesticated in China 42 (Tiwari and Karmakar, 2000). India is also considered as a secondary centre of domestication for 43 soybean (Boyden, 1992 and Khoshoo, 1995).

44 Plant nutrition is a key input to increase the productivity of soybean seed crop. Out of several 45 nutrients provided to plants, phosphorus is a major and essential nutrient for better plant growth and 46 yield as this crop is exhaustive in nature and requires more energy. It is involved in wide range of 47 plant processes from permitting cell division to development of good root system. It stimulates pod 48 setting, seed formation and protein synthesis. The enhancement of symbiotic N-fixation by root 49 nodules as a result of phosphorus nutrition is supported due to high phosphorus requirement of the 50 bacteroides (Kuepper, 2003; Mallarino, 2005).

51 Sulphur has been recognised for over 100 years as one of the essential elements required for 52 the growth of plants. Global reports of sulphur deficiency and consequent crop responses are quite 53 ostensible (Singh and Rath, 1984). Sulphur plays an important role in the synthesis of protein and the 54 essential sulphur containing amino-acids, methionine and cysteine, vitamins and chlorophyll. It is 55 essential for the activation of certain enzymes and is a vital constituent of ferredoxin which participate 56 in the photosynthesis process. Further, without adequate S, crops cannot reach their full potential in 57 terms of yield, quality or protein content nor can they make efficient use of applied N.

58 Soybean plays a vital role in agricultural economy of India. The low productivity of the crop 59 is due to several constraints, one among the important is unbalanced nutrition (Sharma *et al.*, 1991). 60 For its optimum yield realization, it is necessary to optimize the nutrient inputs. Fertilizer application 61 is very important practice and at present the most baffling as well. Soybean shows inconsistent 62 response to application of phosphorus and sulphur. High soybean yield demands high fertilizer 63 dosage, applied directly to the crop or accrued through preceding crops.

64 **Materials and Methods**

65 The experiment was laid out in randomized complete block design with 4 levels of each 66 sulphur and phosphorus and replicated thrice. The corresponding doses of sulphur and phosphorus 67 were applied as per the treatment schedule and mixed thoroughly in the soil. A basal dose consisting

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of uniform recommended dose of nitrogen and potassium was applied through urea 68 and muriate of 69 potash to all plots. At harvest, plants were removed along with pods and the harvested crop of each 70 plot was placed separately on canvas tarpaulin on the concrete floor, sundried and beaten with the 71 stick. The stover was removed and seed winnowed to make it clean. Seed samples were collected 72 treatment wise for analysis. The seed yield obtained from each plot was cleaned, sundried and 73 weighted separately. After harvesting the plants from each plot were tied into separate bundles. The 74 weight of each bundle was recorded and then the stover yield was converted into q ha⁻¹. Seed samples 75 were dried in oven at 65 °C for 48 hours, ground in a Willey Mill and the processed samples were 76 analysed separately. Crude protein content in seed as per the standard method given by Thimmaiah 77 (1999). Oil content in the soybean seed was estimated by ether extraction method using soxhlet 78 apparatus. Triglycerides in soybean were estimated by extraction with alcohol, ethyl ether and 79 petroleum ether and identified by Gas chromatography method of AOAC (2000). Linoleic and 80 lenolenic acid in soybean seeds were estimated by extraction with alcohol, ethyl ether and petroleum 81 ether and identified by Gas chromatography method of AOAC (2000). 2 g of dried and powdered 82 sample was digested first with diluted H₂SO₄ and then with NaOH. Final residue was ashed and 83 expressed in per cent (AOAC, 1975). Carbohydrates in soybean seed were determined as per the 84 standard method given by Thimmaiah (1999). Linoleic and lenolenic acid in soybean seeds were 85 estimated by extraction with alcohol, ethyl ether and petroleum ether and identified by Gas 86 chromatography method of AOAC (2000).

87 Results and Discussion:-

88 The interaction effect of sulphur and phosphorus on zinc, iron and copper content was 89 significant at lower levels of phosphorus. Maximum zinc concentration (117.87 mg kg⁻¹) was 90 recorded in the treatment combination of 45 kg S with 30 kg P₂O₅ ha⁻¹, which was superior over other 91 treatment combinations but at par with 30 kg S and 30 kg P₂O₅ ha⁻¹. However higher amount of 92 manganese content (4.19 mg kg⁻¹) in soybean was recorded with treatment receiving 45 kg S and 90 93 kg P₂O₅ ha⁻¹ in comparison to other lower treatments combinations. However it was at par with 94 treatment combination of S₄₅P₆₀ and S₃₀P₉₀. An increase in the levels of phosphorus and S Sulphur form 95 significantly increased cystine and cysteine content (Table-1). The increase in cystine content due to 96 phosphorus application might be attributed to the improved sulphur and nitrogen nutrition of crop. 97 The increase in cystine content with sulphur application might be due to the fact that sulphur is a main 98 constituents of cystine in amino acid of protein (Meena *et al.*, 2006). Increase in the cysteine content 99 of soybean with phosphorus application was due to improved sulphur and nitrogen nutrition of the 100 crop. These findings were in conformity with those reported by Sentimenla and Singh (2012). 101 Combined application of phosphorus and sulphur increased the cysteine content significantly from 102 0.45 per cent in the control to a maximum of 1.03 per cent at S₄₅P₉₀ treatment combination (Table-1).

Comment [WU4]: Which statistical program did you use? Incorporate both, in the tables and the manuscript, which treatment is statistically significantly different (high or low), or indicate that there's on differences between the treatments.

Comment [WU5]: Here's no method of nutrients determination.

Comment [WU6]: It is not maximum...you should write 'the highest'. Apply this to whole manuscript.

Comment [WU7]: Is this statistical significant? There's no this kind of identification in Table 1.

Comment [WU8]: Not shown in tables!

Comment [WU9]: There's no discussion about sulphur, copper, iron and magnesium. Those four nutrients are shown in table 1.

Comment [WU10]: This is not part of table 1.

Comment [WU11]: Nitrogen fertilization was unique to all treatments!

Comment [WU12]: Nitrogen fertilization was unique to all treatments!

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Table:-2. Effect of phosphorus and sulphur on micro nutrient content in soybean. 196 (Pooled data)

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The significant interaction effect of phosphorus and sulphur on cysteine content was also reported by Sentimenla and Singh (2012).

Methionine content increased significantly, increase in methionine content due to sulphur application might be attributed to the role of sulphur in synthesis of sulphur amino acids like methionine (Lakkineni and Abrol, 1994). Interaction (Table 2) resulting from the combined application of phosphorus and sulphur enhanced the methionine content significantly from 0.40 per cent in the control to a maximum of 0.98 per cent at S₄₅P₉₀ treatment combination. The carbohydrate content of soybean seed increased significantly with the application of phosphorus from 19.11 per cent in the control to 22.99 per cent at 90 kg P₂O₅ ha⁻¹. The increase in carbohydrate content due to phosphorus application might be attributed to the fact that phosphorus plays an important role in photosynthesis as well as energy store. Increase in carbohydrate content of soybean seed with sulphur application may be attributed to the improvement in other nutrients especially potassium which helps in translocation of carbohydrates. A significant increase in carbohydrate content due to combined application of phosphorus and sulphur (interaction) was observed. The carbohydrate content of soybean seed increased significantly from 18.57 per cent in the control to 22.19 per cent with S₄₅P₉₀ treatment combination.

Maximum oil content (Table 3) of 21.24 per cent due to sulphur application was obtained at 45 kg S ha⁻¹ which was significantly superior to control and other treatments. Application of 45 kg S ha⁻¹ increased the oil content by 10.45 per cent over the control. Increased in the oil content of soybean with sulphur application may be due to increase in fatty acids as sulphur is involved in fat metabolism. These findings were in conformity with those reported by Faizani *et al.* (1990) and Shah (2001). The significantly higher oil content with the increasing levels of phosphorus was due to fact that phosphorus is one of the main constituent of the fatty acids. A significant increase in oil content of soybean due to phosphorus application was also reported by Gangadhar *et al.* (1990), Krishnamurthi and Mathan (1996), Sreemannarayana *et al.* (1995).

Triglycerides content of soybean seed increased significantly with increasing levels of sulphur and phosphorus application. The increase in triglycerides content could be attributed to the role of sulphur in synthesis of this saturated oil (Faizani *et al.*, 1990). Interaction resulting from the application of both phosphorus and sulphur increased the triglycerides content significantly from 1.40 per cent in the control to a maximum of 1.98 per cent S₄₅P₉₀ treatment combination which may be attributed to the synthesis of triglycerides in presence of both sulphur and phosphorus. Linoleic acid and linolenic acid is a component of fatty acid and with increase in oil content of soybean there will be increase in linoleic acid content. Enhance oil content due to synthesis of fatty acids as their etherification by accelerating biochemical reactions in glycoxalate cycle (Dwivedi and Bapat, 1998). All the fertilizer treatments increased significantly unsaturated fatty acid with increasing level S.

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Higher percentage of unsaturated fatty acid with nutrient supply may be attributed to 138 balance nutrition
139 of crop (Chaturvedi *et al.*, 2010). Similar results were also reported by Aziz (2011).

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Comment [WU15]: CONCLUSION chapter is missing.

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188 58.

189 **Table:-1. Effect of phosphorus and sulphur on nutrient content in soybean.**
190 **(Pooled data)**

Comment [WU16]: It is not clear why nutrients are shown in different units!!

Table:-2. Effect of phosphorus and sulphur on micro nutrient content in soybean.
196 **(Pooled data)**

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Table:-3. Effect of phosphorus and sulphur on oil and fatty acid content in soybean.
203 **(Pooled data)**

Comment [WU18]: You should revised all 3 table captions...and than table content, and later the references on tables in the manuscript!