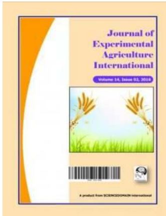

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Effect of Phosphorus and Sulphur on Nutrient and Amino Acids Content of Soybean (*Glycine max* L. Merill) under 'Alfisols'

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Authors' contributions

This work was carried out in collaboration between all authors. Author SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JAW, BAL, AF and PS managed the analyses of the study. Authors SQ, ZAD and NA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at KVK, Srinagar Kashmir, Jammu and Kashmir, India, during two consecutive *kharif* seasons of 2010 and 2011 to study the "Effect of phosphorus and sulphur on yield and quality of soybean (*Glycine max* L. Merill) under Eutrochrepts". The experiment was laid down under 16 treatment combinations viz four levels of phosphorus (0, 30, 60, 90 kg P_2O_5 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 P_2O_5 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, 0.434 and 0.501% 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

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macro as well as micronutrient content except Zn in seed was observed. Combined application of phosphorus and sulphur further enhanced the nutrient content of soybean seed. Combined application of phosphorus and sulphur enhanced the crude protein and oil content in soya seed [1]. Combined application of 45 kg S and 90 kg P_2O_5 ha^{-1} recorded significantly higher carbohydrate content (23.49%) in soybean seed. Increasing levels of phosphorus and sulphur resulted in gradual increase in linoleic (Omega-6) and linolenic acid (Omega-3). Individual as well as interaction effect of P and S was significant in enhancing the sulphur containing amino acids viz., cystine cystein and methionine content of soybean seed.

Keywords: Phosphorus; sulphur; soybean; Zn; Fe; Cu; Oil; fatty acids; cystine cystein and methionine.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] has become the miracle crop of the 21st century. It belongs to the family Leguminosae, sub-family Papilionaceae and the genus Glycine. It is a triple beneficiary crop, which contains about 40 per cent protein, possessing high level of essential amino-acids methionine and cystine, 20 per cent oil rich in poly unsaturated fatty acids especially omega-6 and omega-3 fatty acids, 6-7 per cent total minerals, 5-6 per cent crude fibre and 17-19 per cent carbohydrates [1]. Besides, it has good amount of iron, vitamin B-complex and isoflavones such as daidzein, genistein of clycitin. Presence of calcium and iron makes it highly suitable for women who suffer from osteoporosis and anaemia. The isoflavones of soybean have been found to possess health benefits, as they exhibited properties like cancer preventing, combating menopausal problem and helping to recover from diabetes [2]. At present, India ranks fifth with respect to soybean acreage and production in the globe. In the past three decades from 70s onwards, the crop exhibited a phenomenal growth. In India, the planted area under soybean in 2010-11 was above 9.3 M ha, which produced about 10.4 MT of soybean with a productivity of 1.2 t ha-1.

Soybean was considered only as a food and fodder crop till World War-II when its potential as an oilseed crop was realized. Due to its multifaceted uses, soybean has since progressed by leaps and bounds as an oilseed crop. So much so that on the global scale it has come to the top of the list of oilseed crops and contributes over one-third of the total supply of the world vegetable oil pool. Indians as such, know soybean since ages as it was in cultivation in northern and north-eastern hills as food plant and is a part of routine diet of the people [3]. Black-seeded soybean has been grown since early times in the northern and north eastern hills and in scattered area in the central part of the country. Soybean was introduced in India probably as soon as it was domesticated in China [4]. India is also considered as a secondary centre of domestication for soybean [5,6].

Plant nutrition is a key input to increase the productivity of soybean seed crop. Out of several nutrients provided to plants, phosphorus is a major and essential nutrient for better plant growth and yield as this crop is exhaustive in nature and requires more energy. It is involved in wide range of plant processes from permitting cell division to development of good root system. It stimulates pod setting, seed formation and protein synthesis. The enhancement of symbiotic N-fixation by root nodules as a result of phosphorus nutrition is supported due to high phosphorus requirement of the bacteroides [7,8].

Sulphur has been recognised for over 100 years as one of the essential elements required for the growth of plants. Global reports of sulphur deficiency and consequent crop responses are quite ostensible [9]. Sulphur plays an important role in the synthesis of protein and the essential sulphur containing amino-acids, methionine and cysteine, vitamins and chlorophyll. It is essential for the activation of certain enzymes and is a vital constituent of ferrodoxin which participate in the photosynthesis process. Further, without adequate S, crops cannot reach their full potential in terms of yield, quality or protein content nor can they make efficient use of applied N.

Soybean plays a vital role in agricultural economy of India. The low productivity of the crop is due to several constraints, one among the important is unbalanced nutrition. For its optimum yield realization, it is necessary to optimize the nutrient inputs. Fertilizer application is very important practice and at present the most baffling as well. Soybean shows inconsistent response to application of phosphorus and sulphur. High soybean yield demands high fertilizer dosage, applied directly to the crop or accrued through preceding crops. Thus present study was undertaken to assess the importance of sulphur and phosphorus on nutrient and amino acids content of soybean.

2. MATERIALS AND METHODS

The experiment was laid out in randomized complete block design with 4 levels of each sulphur and phosphorus and replicated thrice. The corresponding doses of phosphorus (0, 30, 60, 90 kg P_2O_5 ha^{-1}) and four levels of sulphur 0, 15, 30, 45 kg S ha^{-1} were applied as per the treatment schedule and mixed thoroughly in the soil at the time of soil preparation. A basal dose consisting of uniform recommended dose of nitrogen and potassium was applied through urea and muriate of potash to all plots. At harvest, plants were removed along with pods and the harvested crop of each plot was placed separately on canvas tarpaulin on the concrete floor, sundried and beaten with the stick. The stover was removed and seed winnowed to make it clean. Seed samples were collected treatment wise for analysis. The seed yield obtained from each plot was cleaned, sundried and weighted separately. After harvesting the plants from each plot were tied into separate bundles. The weight of each bundle was recorded and then the stover yield was converted into q ha^{-1}. Seed samples were dried in oven at 65℃ for 48 hours, ground in a Willey Mill and the processed samples were analysed separately. Crude protein content in seed as per the standard method given by [10]. Oil content in the soybean seed was estimated by ether extraction method using soxlet apparatus. Triglycerides in soybean were estimated by extraction with alcohol, ethyl ether and petroleum ether and identified by Gas chromatography method [11]. Linoleic and lenolenic acid in soybean seeds were estimated by extraction with alcohol, ethyl ether and petroleum ether and identified by Gas chromatography method. Two gram of dried and powdered sample was digested first with diluted H_2SO_4 and then with NaOH. Final residue was ashed and expressed in per cent. Carbohydrates in soybean seed were determined as per the standard method given by [12]. Linoleic and lenolenic acid in soybean seeds were estimated by extraction with alcohol, ethyl ether and petroleum ether and identified by Gas chromatography method of [13]. Statistical analysis was carried out using MINITAB.

3. RESULTS AND DISCUSSION

The interaction effect of sulphur and phosphorus on zinc, iron and copper content was significant at lower levels of phosphorus. Maximum zinc concentration (117.87 mg kg^{-1}) was recorded in the treatment combination of 45 kg S with 30 kg P_2O_5 ha^{-1}, which was superior over other treatment combinations but at par with 30 kg S and 30 kg P_2O_5 ha^{-1}. However higher amount of manganese content (4.19 mg kg^{-1}) in soybean was recorded with treatment receiving 45 kg Sulphur and 90 kg P_2O_5 ha^{-1} in comparison to other lower treatments combinations. However it was at par with treatment combination of $S_{45}P_{60}$ and $S_{30}P_{90}$.An increase in the levels of phosphorus and Sulphur form significantly increased cystine and cysteine content (Table-1). The increase in cystine content due to phosphorus application might be attributed to the improved sulphur and nitrogen nutrition of crop. The increase in cystine content with sulphur application might be due to the fact that sulphur is a main constituents of cystine in amino acid of protein [17]. Increase in the cysteine content of soybean with phosphorus application was due to improved sulphur and nitrogen nutrition of the crop. Combined application of phosphorus and sulphur increased the cysteine content significantly from 0.45 per cent in the control to a maximum of 1.03 per cent at $S_{45}P_{90}$ treatment combination (Table-1).

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 [14]. 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 $S_{45}P_{90}$ 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 P_2O_5 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 $S_{45}P_{90}$ treatment combination.

Table 1. Effect of phosphorus and sulphur on nutrient content in soybean (Pooled data)

Treatment		Zn content (mg / kg)	Fe content (mg / kg)	Cu content (mg / 100g)	Mg content (%)	S content (%)	Cu content (mg /kg)	Mn content (mg/ kg)
Phosphorus 0 Kg/ha	S_0	76.44	6.38	0.72	0.380	0.401	0.72	1.81
	S_{15}	85.75	7.69	1.55	0.399	0.429	1.55	2.52
	S_{30}	91.12	8.39	1.89	0.407	0.458	1.89	2.86
	S_{45}	94.07	8.61	1.99	0.412	0.490	1.99	2.99
Phosphorus 30 Kg/ha	S_0	100.24	7.63	1.51	0.395	0.407	1.51	2.52
	S_{15}	109.55	8.91	2.35	0.415	0.436	2.35	3.21
	S_{30}	114.92	9.61	2.69	0.423	0.467	2.69	3.56
	S_{45}	117.87	9.83	2.79	0.428	0.498	2.79	3.69
Phosphorus 60 Kg/ha	S_0	94.46	8.36	1.90	0.410	0.415	1.90	2.89
	S_{15}	103.77	9.64	2.76	0.431	0.442	2.76	3.60
	S_{30}	109.14	10.34	3.10	0.439	0.475	3.10	3.93
	S_{45}	112.09	10.56	3.00	0.445	0.505	3.00	4.06
Phosphorus 90 Kg/ha	S_0	90.32	8.66	2.03	0.423	0.421	2.03	3.00
	S_{15}	99.63	9.93	2.87	0.439	0.449	2.87	3.73
	S_{30}	105.00	10.63	3.30	0.447	0.483	3.30	4.06
	S_{45}	107.95	10.85	3.21	0.452	0.513	3.21	4.19
$CD_{(p\leq0.05)}$ (P)		1.86	0.22	0.08	0.001	0.002	0.08	0.13
(S)		1.86	0.41	0.09	0.003	0.003	0.09	0.11

S_0 = Control, S_{15} = 15 kg Sulphur ha^{-1}, S_{30} = 30 kg Sulphur ha^{-1}, S_{45} = 45 kg Sulphur ha^{-1}

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

Treatment		Mn content (mg /kg)	Crude protein content (%)	Cystine content (%)	Cysteine content (%)	Methonine content (%)	Fibre content (%)	Carbohydrate content (%)
Phosphorus 0 Kg/ha	S_0	1.81	35.23	0.31	0.45	0.40	5.03	18.57
	S_{15}	2.52	36.69	0.48	0.60	0.55	4.99	18.97
	S_{30}	2.86	38.05	0.67	0.76	0.70	4.96	19.28
	S_{45}	2.99	38.96	0.84	0.90	0.85	4.90	19.61
Phosphorus 30 Kg/ha	S_0	2.52	37.16	0.34	0.50	0.44	4.85	21.28
	S_{15}	3.21	38.60	0.53	0.64	0.59	4.81	21.68
	S_{30}	3.56	39.98	0.71	0.80	0.74	4.78	21.99
	S_{45}	3.69	40.89	0.88	0.94	0.89	4.72	22.32
Phosphorus 60 Kg/ha	S_0	2.89	38.15	0.39	0.54	0.48	4.77	22.30
	S_{15}	3.60	39.62	0.58	0.69	0.63	4.73	22.70
	S_{30}	3.93	40.98	0.76	0.84	0.77	4.70	23.01
	S_{45}	4.06	41.89	0.93	0.98	0.93	4.64	23.34
Phosphorus 90 Kg/ha	S_0	3.00	38.20	0.44	0.57	0.51	4.74	22.45
	S_{15}	3.73	39.65	0.63	0.72	0.67	4.70	22.85
	S_{30}	4.06	41.01	0.80	0.87	0.81	4.67	23.16
	S_{45}	4.19	41.92	0.98	1.03	0.98	4.61	23.49
$CD_{(p\leq0.05)}$ (P)		0.13	0.21	0.01	0.67	0.01		0.07
(S)		0.11	0.35	0.01	0.72	0.02		0.09

S_0 = Control, S_{15} = 15 kg Sulphur ha^{-1}, S_{30} = 30 kg Sulphur ha^{-1}, S_{45} = 45 kg Sulphur ha^{-1}

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

Treatment		Oil content (%)	Triglycerides (Steric acid) content (%)	Linoleic acid (omega 6) content (%)	Linolenic acid (omega -3) content (%)
Phosphorus 0 Kg/ha	S_0	17.85	1.40	51.01	6.17
	S_{15}	19.00	1.57	51.50	6.25
	S_{30}	19.62	1.72	52.71	6.96
	S_{45}	20.08	1.88	52.88	7.35
Phosphorus 30 Kg/ha	S_0	18.88	1.44	51.21	6.35
	S_{15}	20.02	1.60	52.37	6.65
	S_{30}	20.64	1.76	52.87	7.07
	S_{45}	21.10	1.91	53.04	7.44
Phosphorus 60 Kg/ha	S_0	19.50	1.49	52.20	6.48
	S_{15}	20.66	1.64	53.35	6.76
	S_{30}	21.28	1.80	53.85	7.16
	S_{45}	21.74	1.94	54.02	7.56
Phosphorus 90 Kg/ha	S_0	19.82	1.53	52.50	6.54
	S_{15}	20.96	1.68	53.68	6.87
	S_{30}	21.58	1.84	54.18	7.24
	S_{45}	22.04	1.98	54.35	7.68
$CD_{(p\leq0.05)}$ (P)		0.14	0.01	0.11	0.03
(S)		0.11	0.01	0.14	0.05

S_0 = Control, S_{15} = 15 kg S ha^{-1}, S_{30} = 30 kg S ha^{-1}, S_{45} = 45 kg S ha^{-1}

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 [15,16]. 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 [17,18,19].

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 [20]. 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 $S_{45}P_{90}$ 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 [21]. All the fertilizer treatments increased significantly unsaturated fatty acid with increasing level S. Higher percentage of unsaturated fatty acid with nutrient supply may be attributed to balance nutrition of crop [22]. Similar results were also reported by [23].

4. CONCLUSION

Phosphorus and sulphur application resulted in a significant increase in the nutrient concentration of macro as well as micronutrients in seed. On the application of 90 kg P2O5 ha-1, Fe, Cu, Mn was 100.01, 2.86 and 3.74 mg kg-1, respectively. At higher levels of phosphorus application, Zn content of seed decreased and it was highest at 30 kg P_2O_5 ha-1 .With application of 45 kg S ha-1, the content of Mg and S was 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 except Zn in seed was observed. Combined application of phosphorus and sulphur further enhanced the nutrient content of soybean seed. Highest triglycerides, methionine, and manganese content can be achieved at Sulphur 45 kg/ha and phosphorus @90 kg/ha however Maximum zinc concentration was recorded in the treatment combination of 45 kg S with 30 kg P_2O_5 ha^{-1}, 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 at Sulphur 45 kg/ha and phosphorus @90 kg/ha which may be attributed to the synthesis of triglycerides in presence of both sulphur and phosphorus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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