

AGRONOMIC BIOFORTIFICATION OF GREEN BEAN (*PHASELOUS VULGARIS* L.) WITH ELEMENTAL SULPHUR AND FARMYARD MANURE

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Abstract. The effects of elemental sulphur and farmyard manure on agronomic biofortification within the parameters of N, P, S and N:S ratio were evaluated in green bean (*Phaseolus vulgaris* L.). Sulphur 0 (S₀), 50 (S₁), 100 (S₂), 150 (S₃), 200 (S₄), 400 (S₅) mg kg⁻¹ and farmyard manure 0 (FYM₀), 3 (FYM₁) tonnes da⁻¹ were applied to a calcareous sandy loam soil with low organic matter and sulphur deficient. Three weeks after the applications, bean seeds were sown in pot soils and after eight weeks of growing period the shoots were harvested. The soil pH was decreased while EC was increased by the applications of S and FYM. The P concentration of shoot was increased by S with FYM. While the dry weight and S concentration of shoot were increased, N concentration was slightly decreased by S, alone. The N:S ratio decreased from 23.76 in S₀FYM₀ to 15.93 in S₅FYM₁. All results indicate that sulphur applications in S₁ and S₂ levels with farmyard manure can be sufficient for growing bean in the soil.

Keywords: *malnutrition, chemical fertilizer, organic manure, nitrogen, phosphorus*

Introduction

Two thirds of the world population faces with one or more essential mineral deficiencies in their nutrition. Solutions to this problem are being investigated by means of dietary diversification, mineral adding, food supplements or increasing bioavailability and concentrations of mineral elements during crop production. Especially there have been recent studies on plants which have an essential importance in human nutrition with agronomic and genetic biofortification. Agronomic biofortification tries to ensure the optimization of mineral fertilizer applications and improving of mineral element mobility and solubility in soil. The optimization of chemical fertilizer and organic manure applications or the applications of essential elements with enriched fertilizer (especially NPK fertilizers) throughout soil, leaf or seed during cultivation should not only provide a quick solution in human and animal nutrition but also will be a complementary approach for ongoing plant breeding programme.

Sulphur is one of the essential elements in plant growth. Plants need sulphur for protein synthesis, chlorophyll, oil and vitamin formation. Sulphur deficiency has a negative effect on both yield and quality of crops which are grown for human and animal consumption (Tiwari et al., 1997). The specific aim of most biofortification studies is to reduce mineral, vitamin and protein deficiencies in edible parts of plants. Sulphur takes role in synthesis of some compounds such as cysteine, cystine and methionine for protein formation in plants. Low sulphur content in proteins is a nutritionally adverse situation and the methionine which is the main source of protein

and can be synthesized in the presence of sulphur is a basic amino acid for human nutrition.

Sulphate absorbed by the roots is the most important source of sulphur. Despite the sulphur's distribution on all parts of soil profile, it has at its highest concentration on surface soil which is rich in organic matter. Sandy or low organic content soils are the ones which are encountered with sulphur deficiency. On the situations of high efficiency on crop yield, especially on sandy soils and soils that are poor in organic matter which has abundant watering, the sulphur content is not in sufficient quantity for the needs of plants (Anonymous, 1982). The sulphur deficiency in plants can also occur on coarse textured soils having a high pH and when there is insufficient moisture on their root zone (Tiwari, 1995). And also the sulphur deficiency usually occurs on cultivation areas where chemical fertilizers are used and of the plants that are in highly need of sulphur due to their high yield capacity. Therefore, in addition to nutrients such as N, P₂O₅ and K₂O, S which is also named as the fourth basic macro nutrient should also be considered while doing fertilization programmes.

Bean is not only a product of high nutritional value, but also is an important vegetable protein source for humans. It has been widely consumed in Turkey and in Asian, African, European, North, South and Central American countries. For this reason, it is essential that it takes an important role among plants which are to be studied for increase yield and nutritive value. In this study, the effect of sulphur and farmyard manure applications on calcareous sandy loam soil having a low-sulphur and organic matter content for agronomic biofortification in bean is being examined using parameters of N, P, S and N:S.

Material and methods

Experimental setup

Soil was air dried and passed through a 4 mm sieve. A total of 9 kg of sieved soil was placed in pots with holes at the bottom. Sulphur (S₀:0, S₁:50, S₂:100, S₃:150, S₄:200, S₅:400 mg kg⁻¹) without farmyard manure (FYM₀: 0 tonnes da⁻¹) and with farmyard manure (FYM₁: 3 tonnes da⁻¹) was applied to soil according to completely randomized design factorial with 4 replicates. At the time of the application materials were mixed to soil, soil samples were taken at 1st sampling period. Then, basal fertilization (as 8 kg N da⁻¹, 8 kg P₂O₅ da⁻¹ and 8 kg K₂O da⁻¹) using 15.15.15 fertilizer was made to each pot soil. Each pot was equally watered and incubated for 3 weeks. The green bean 'Öz Ayşe' was used as an experimental plant. At the end of the 3 weeks, 2nd period soil samples were taken and four green bean seeds were sown in each pot soil. After the seeds germination, one bean plant was left in per pot and the plants were grown for 8 weeks. During the vegetation period, N 8.5 kg da⁻¹ (NH₄NO₃, 33% N), P₂O₅ 3.7 kg da⁻¹ (MAP), K₂O 10 kg da⁻¹ (KNO₃), MgO 0.62 kg da⁻¹ (MgNO₃) and 1.73 kg microelement fertilizer da⁻¹ (Hortrilon, 5% Fe, 2.5% Mn, 0.5% Zn, 2.5% Cu) were applied to each pot as fertilizer solution. At the end of the experiment, the shoots were harvested and 3rd period soil samples were taken from each pot.

Plant analysis

The shoots were washed with deionized water and then dried 65 °C for 72 h to determine dry weight. The dried shoots were ground in a stainless steel mill which

enabled them to be passed through a 20 mesh screen. The samples of 0.5 g each were digested with 10 mL HNO₃ and HClO₄ (4:1) acid mixture on a hot plate and filtered and then, diluted to 100 mL using distilled water. Total S was conducted by the turbidimetric method with BaCl₂·2H₂O and the readings were taken using a spectrophotometer at 430 nm (Kacar and Inal, 2008). Total P was measured by a modified colorimetric molybdo-vanado-phosphate method using a spectrophotometer at 430 nm (Kacar and Inal, 2008). Total N was determined by a modified Kjeldahl procedure (Kacar and Inal, 2008).

Soil analysis

The soil samples were air-dried and passed through a 2 mm sieve. The pH was measured in H₂O (1:2.5 soil:deionized water) and the electrical conductivity (EC) value was determined directly on the saturation paste. The soil particle size analysis was done by using the hydrometer method (Bouyoucos, 1955) and the CaCO₃ content was determined by using a Scheibler calcimeter. Organic matter was determined by using modified Walkley-Black procedure (Black, 1965). The total nitrogen was done by using modified Kjeldahl procedure (Kacar, 2009). Available P was extracted by NaHCO₃ and determined by a molybdate colorimetric method (Olsen and Sommers, 1982). Soil samples were extracted for SO₄-S by using 500 mg kg⁻¹ P as KH₂PO₄ which contents of Fox et al. (1964). Analyses were conducted by the turbidimetric method with BaCl₂·2H₂O and the readings were taken using a spectrophotometer at 430 nm (Kacar, 2009). Some of the physical and chemical properties of experiment soil were given in *Table 1*.

Table 1. Physical and chemical properties of soil used

Parameter	Soil
Sand (%)	63.52
Clay (%)	18.48
Silt (%)	18
Texture	Sandy loam
pH	7.80
CaCO ₃ (%)	18.92
EC (dS m ⁻¹)	2.07
Organic matter (%)	2.27
Total N (%)	0.049
Available-P (mg kg ⁻¹)	34.57
Extractable-SO ₄ -S (mg kg ⁻¹)	2.40

Statistical analysis

The data were analyzed by standard ANOVA procedures and their significances were always based on the $P < 0.05$ level using the LSD tests.

Results and discussion

Effects of sulphur and farmyard manure on soil pH and EC

In the 1st soil sampling period, the farmyard manure caused an important but little increase of 0.03 units on soil pH. In the 2nd and 3rd soil sampling periods, the effects of interactions of sulphur and farmyard manure on soil pH were found significant. In the 2nd sampling period, the highest decrease on soil pH was respectively obtained as 7.65 and 7.64 in applications of S₅FYM₀ ve S₅FYM₁. When the sulphur applications whether with or without farmyard manure compared, the applications of S₀FYM₀ and S₀FYM₁, S₂FYM₀ and S₂FYM₁, S₄FYM₀ and S₄FYM₁ differed from each other and in these applications, soil pH was determined to be a little bit lower when sulphur is applied with farmyard manure. And in the 3rd soil sampling periods, the highest decrease on soil pH was respectively obtained as 7.70 and 7.76 in applications of S₅FYM₀ and S₅FYM₁. When the sulphur applications with or without farmyard manure compared, S₂FYM₀ and S₂FYM₁, S₅FYM₀ and S₅FYM₁ differed from each other and in these applications, a little increase in soil pH was obtained when sulphur is applied with farmyard manure. In an overall evaluation, especially in the 2nd sampling period (after three weeks from applications) depending on irrigation, the pH of control soil (S₀FYM₀) rose from 7.81 up to 8.01, whereas no increase in sulphur with farmyard manure applications was observed. Almost similar effects of the applications were determined in the 3rd sampling period (Table 2). Kaplan and Orman (1998) reported that the sulphur applied to high calcareous soil decreased pH in units of 0.07-0.35 and the pH started to rise again depending on time.

Table 2. *Effects of sulphur (S) and farmyard manure (FYM) on soil pH*

Sulphur doses	Soil pH								
	1 st soil sampling			2 nd soil sampling			3 rd soil sampling		
	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means
S ₀	7.81	7.83	7.82	8.01 a ⁽¹⁾ , A	7.88 a, B	7.95	7.89 a, A	7.90 a, A	7.89
S ₁	7.85	7.86	7.85	7.83 b, A	7.85 a, A	7.84	7.81 b, A	7.83 b, A	7.82
S ₂	7.83	7.87	7.85	7.83 b, A	7.74 b, B	7.79	7.77 c, B	7.80 c, A	7.78
S ₃	7.81	7.87	7.84	7.74 c, A	7.75 b, A	7.75	7.78 c, A	7.79 c, A	7.78
S ₄	7.83	7.85	7.84	7.76 c, A	7.72 b, B	7.74	7.76 c, A	7.75 d, A	7.76
S ₅	7.83	7.87	7.85	7.65 d, A	7.64 c, A	7.64	7.70 d, B	7.76 d, A	7.73
Means	7.83 B ⁽²⁾	7.86 A		7.80	7.76		7.78	7.80	
ANOVA ⁽³⁾									
S	n.s.			**			**		
FYM	**			**			**		
S*FYM	n.s.			**			*		

⁽¹⁾Means in the same column followed by the same letter are not significantly different at 5% probability level by LSD test; ⁽²⁾Means in the same row followed by the capital and bold same letter are not significantly different at 5% probability level by LSD test; ⁽³⁾Significance levels: *p<0.05; **p<0.01; n.s.: non significant

In the 1st soil sampling period, the effects of sulphur on soil EC was not significant whereas the farmyard manure effect was important. While the soil EC was highly affected from the sulphur and farmyard manure in the 2nd and 3rd sampling periods, the

interaction between these two applications was not significant in each three sampling periods. The farmyard manure caused an increase about 0.29, 0.37 and 0.29 units in soil EC, respectively in the 1st, 2nd and 3rd sampling periods. The sulphur yet caused an increase on soil EC only in the 2nd and 3rd sampling periods and the highest increase of EC was observed in S₄ and S₅ (Table 3). The sulphur applications are reported to cause an increase in soil salinity in several studies (Modaish et al., 1989; Kaplan and Orman, 1998; Orman and Kaplan, 2011; Orman and Ok, 2012). And also in this study, it has been indicated that especially the sulphur applications increases the soil salinity significantly. It is thought that this is because of the SO₄⁻² having been formed in soil after the sulphur applications and also because of irrigation and fertilization during the growing period. Thus, a significantly positive correlation between EC values and SO₄⁻²-S concentrations of greenhouse soils where tomato cultivation is made in West Mediterranean Region has been reported by Orman and Kaplan (2009).

Table 3. Effects of sulphur (S) and farmyard manure (FYM) on soil EC

Sulphur doses	Soil EC (dS m ⁻¹)								
	1 st soil sampling			2 nd soil sampling			3 rd soil sampling		
	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means
S ₀	2.10	2.33	2.22	4.35	5.01	4.68 c ⁽¹⁾	3.45	3.61	3.53 d
S ₁	2.26	2.55	2.41	5.20	5.85	5.52 b	4.05	4.05	4.05 c
S ₂	2.25	2.50	2.38	5.26	5.61	5.44 b	4.26	4.32	4.29 bc
S ₃	2.13	2.49	2.31	5.34	5.55	5.44 b	4.07	4.26	4.16 c
S ₄	2.20	2.55	2.37	5.77	6.23	6.00 a	4.07	5.00	4.53 ab
S ₅	2.17	2.49	2.33	6.43	6.34	6.38 a	4.64	5.02	4.83 a
Means	2.22 B ⁽²⁾	2.48 A		5.39 B	5.76 A		4.09 B	4.38 A	
ANOVA ⁽³⁾									
S	n.s.			**			**		
FYM	**			**			**		
S*FYM	n.s.			n.s.			n.s.		

⁽¹⁾Means in the same column followed by the same letter are not significantly different at 5% probability level by LSD test; ⁽²⁾Means in the same row followed by the capital and bold same letter are not significantly different at 5% probability level by LSD test; ⁽³⁾Significance levels: **p<0.01; n.s.: non significant

Effects of sulphur and farmyard manure on bean plant

The effects of applications on total nitrogen, sulphur, and phosphorus concentrations, N:S ratio and dry weight in the shoots of bean were given in Table 4.

The sulphur alone is found important to have a significant effect on nitrogen concentration in shoots an especially in higher doses of 100 mg S kg⁻¹, there has been a reduction in nitrogen concentration. Schung (1990) specified that the increase of S content in plant tissues with suitable sulphur fertilization in vegetables had been effective in decrease of NO₃ content in tissues. It was reported that sulphur applied to soil has no significant effect on nitrogen concentration in tomato plant, but the N concentration is observed to be lower than the control (Orman and Kaplan, 2011). Gaines and Phatak (1982) reported that with the increase of the sulphur ratio applied to soybean grown in hydroponic culture, the N concentration decreased in a significant

rate. In our study, the decrease in nitrogen concentrations of bean shoots by the sulphur applications can either be related with sulphur and nitrogen relationships, or just arise due to the dilution effect of nitrogen in result of dry weight increase by sulphur.

Table 4. Effects of sulphur (S) and farmyard manure (FYM) on shoot N, S, P concentrations, N:S ratio and dry weight

Sulphur doses	N (%)			S (%)			P (%)			N:S			Dry weight (g pot ⁻¹)		
	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means	FYM ₀	FYM ₁	Means
S ₀	3.24	3.22	3.23ab ⁽¹⁾	0.14	0.17	0.16c	0.28c, B ⁽²⁾	0.32a, A	0.30	23.76a, A	18.72bc, B	21.24	21.56	23.01	22.28b
S ₁	3.30	3.25	3.27ab	0.17	0.17	0.17bc	0.34a, A	0.33a, A	0.33	19.87b, A	19.82b, A	19.84	28.61	28.54	28.57a
S ₂	3.42	3.33	3.37a	0.19	0.19	0.19a	0.31b, B	0.34a, A	0.33	18.83bc, A	17.25bcd, A	18.04	28.76	24.72	26.74a
S ₃	3.00	3.22	3.11b	0.18	0.17	0.18ab	0.31b, A	0.32a, A	0.32	16.53cd, A	19.03bc, A	17.78	27.17	28.99	28.08a
S ₄	3.12	3.27	3.20ab	0.19	0.19	0.19a	0.33ab, A	0.33a, A	0.33	16.63cd, A	17.22bcd, A	16.93	26.13	27.44	26.78a
S ₅	3.15	3.09	3.12b	0.19	0.20	0.19a	0.35a, A	0.33a, A	0.34	16.85cd, A	15.93d, A	16.39	27.36	27.96	27.66a
Means	3.20	3.23		0.17	0.18		0.32	0.33		18.74	17.99		26.60	26.78	

ANOVA⁽³⁾

S	*	**	**	***	**
FYM	n.s.	n.s.	*	n.s.	n.s.
S*FYM	n.s.	n.s.	**	**	n.s.

⁽¹⁾Means in the same column followed by the same letter are not significantly different at 5% probability level by LSD test; ⁽²⁾Means in the same row followed by the capital and bold same letter are not significantly different at 5% probability level by LSD test; ⁽³⁾Significance levels: ** p<0.01; n.s.: non significant

The sulphur, farmyard manure and sulphur with farmyard manure interaction had a great impact on shoot phosphorus concentration. The shoot phosphorus concentration is determined as 0.28% in S₀FYM₀ whereas it is seen as 0.35% in S₅FYM₀. Having been evaluated with or without farmyard manure, the applications of S₀FYM₀ and S₀FYM₁, S₂FYM₀ and S₂FYM₁ differed from each other statistically and in these applications, applying sulphur with farmyard manure caused an increase in shoot phosphorus concentration. Also, it is thought that shoot phosphorus concentration increases due to the decrease in soil pH because of consequent sulphur applications. Brahim et al. (2017) indicated that the combined application of rock phosphate and elemental sulphur was the potential to improve the P nutrition of soybean.

The applications of sulphur alone significantly affected on shoot sulphur concentration. There has been an increase between 5.9% and 15.79% in shoot sulphur concentration according to the S₀. This impact is especially determined in 100 mg S kg⁻¹ level. Even though the shoot sulphur concentration has an increase about 5.56% by farmyard manure application, this impact is not found important statistically. Tiecher et al. (2012) observed that the amount of S accumulated in the shoots of sunflower, bean, soybean and castor bean and the level of available S increased due to S fertilization.

Reuther and Robinson (1998) notified that bean plant in early stage has a sufficient S content of 0.16%-0.64% in shoots. The sulphur concentrations of shoots in our study range from 0.14% to 0.20%. According to the limit values, it is significantly output that

there has been an improvement in sulphur nutrition level of bean, especially as a result of sulphur applications.

The sulphur alone and sulphur with farmyard manure applications were significantly affected on total N: total S ratio of shoot. The N:S ratio in shoot is assigned as 23.76 in S_0FYM_0 and 18.72 in S_0FYM_1 and it showed statistically significant differences. The other applications of sulphur with or without farmyard manure were statistically included in the same group. The highest ratio of N:S was determined in S_0FYM_0 application and the lowest of that was in S_5FYM_1 application. The shoot N:S ratio showed a significant reduction especially as a result of sulphur alone applications. Barczak and Nowak (2015) reported that the potato plants fertilised with sulphur, in comparison with the control, N:S ratio was narrowed due to a higher increase in the content of sulphur rather than nitrogen in potato tubers.

Although it has been obtained that the effects of sulphur with farmyard manure has a great role on the shoots N:S ratio, it is thought that the determining factor in the decline of this ratio is the sulphur applications. Total N: Total S ratio of the leguminous for protein synthesis is reported to be 17 (Kacar and Katkat 2007). It is pointed out by Stewart and Porter (1969) that the sulphur deficiency may be limiting in protein formation in case of the total N:total S ratio's being over 16 and it is just a severe sulphur deficiency in case of the ratio's being over 20 or more. The soil SO_4^{2-} -S concentration below 10 mg kg^{-1} is considered to be deficient (Radish et al., 1995). According to this limit value, the extractable SO_4^{2-} -S (2.40 mg kg^{-1}) concentration of our experiment soil appears to be deficient. In this case, N:S ratio designated as 23.76 in S_0FYM_0 application is the indicator of the plant's facing with a deficiency in its sulphur nutrition and this will obviously cause negative effects in protein synthesis. However, by the increase of the sulphur applications, the imbalance of plant's N:S ratios disappeared and it seems to have a balance between nitrogen and sulphur elements which are highly important in protein synthesis. The uptake of sulphur, yield, quality and optimizing the N:S ratio in plants were improved by sulphur fertilization (Skwierawska et al., 2016). But also while trying to maintain this balance, the risk of sulphur's increasing soil salinity should be kept in mind and it should be taken into consideration while doing sulphur fertilization especially on saline soils. A greenhouse survey study in Antalya/Turkey, it appears that there are imbalance for N:S ratio, even if a sulphur deficiency is not determined in tomato leaves (Orman and Kaplan, 2009)

The effect of sulphur alone is found to be important on shoot dry weight. The dry weight was 22.28 g pot^{-1} in S_0 application whereas there became an increase about 17-22% by sulphur applications. The increase occurred was not due to the increase of sulphur level, the sulphur application doses statistically took place in the same group. Crusicol et al. (2006) indicated that sulphur fertilization has not considered by bean (*Phaseolus vulgaris* L.) growers. Whereas, the increase of grain yield can be limited by low levels of sulphur fertilization in common bean crops with high-input technology. Researchers reported that leaf S content, dry weight and grain yield of common bean is increased by sulphur fertilization. Kaplan and Orman (1998) reported an increase in dry weight of sorghum with sulphur applications.

Conclusion

The sulphur is an advisable material in order to decrease the soil pH on calcareous sandy loam and high pH soils and also to increase the uptake of the phosphorus element

by plants of which the solubility in soil is significantly depending on pH. But the risk of the sulphur's creating salinity in soil should be taken into consideration and this negative effect should be well managed especially on the agricultural lands under the risk of salinity. Also, Gulmezoglu et al. (2016) reported that salt application generally reduced the fresh and dry weights of green bean genotypes.

On the sulphur deficient soils, the sulphur or other sulphur fertilizers absolutely should be included on fertilization programmes to obtain sulphur for the plants that are in need of sulphur requirement such as bean. Because it has been determined that if the necessary sulphur applications are not done on such soils, a significant imbalance may emerge in N:S ratio of the plant and the balance may be damaged against sulphur. When considering not only nitrogen but also sulphur as well is effective in protein formation in plants, this situation might have negative repercussions on high protein content plant's protein synthesis and quality such as bean.

According to the data obtained from this study, the applications of sulphur in 50 mg kg⁻¹ and 100 mg kg⁻¹ levels to bean growing on a calcareous sandy loam soils which are deficient in organic matter and SO₄⁻² are thought to be sufficient in terms of yield and quality. Consequently, the further studies are needed to optimize amount of sulphur addition for other soil types and plant species.

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