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Effects of Vanadium on the growth, chlorophyll, and mineral content of Soybean (*Glycine max* (L.) Merr)

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Abstract

The effect of increasing levels of vanadium (0, 0.5, 1, and 2 mg V kg^{-1}) and farmyard manure (FYM) (0, 1 and 2 ton ha^{-1}) on the fresh and dry weight, chlorophyll and mineral content of soybean (*Glycine max* (L.) Merr) was investigated under the greenhouse conditions. Phenologic observations showed that V concentrations were not toxic to the growing plants. Fresh and dry weights of the plants were decreased by applying V or FYM doses while both V and FYM treatments increased the plant growth. Nodule number was only affected by applications of 1 mg kg^{-1} V and 1 ton ha^{-1} FYM doses. Increasing V concentrations caused increases in chlorophyll contents and fresh and dry weights of plants. On the other hand both V and FYM treatments had similar effects on soybean.

Key words: Chlorophyll; soybean; vanadium.

Efectos de vanadio sobre desarrollo, clorofila y el contenido mineral de la soya (*Glycine max* (L.) Merr)

Resumen

El efecto de incrementar los niveles de vanadio (0; 0,5 1 y 2 mg kg^{-1}) y de abono animal (0,1 y 2 ton ha^{-1}) en los pesos fresco y seco de la clorofila y el contenido mineral de la soya (*Glycine max* (L.) Merr) fue investigado bajo condiciones de invernadero. Observaciones fenológicas mostraron que las concentraciones de vanadio no tuvieron ningún efecto tóxico en las plantas en crecimiento. Los pesos secos y frescos de las plantas decrecieron cuando se aplicaban dosis de vanadio o abono animal, mientras que tratamientos con ambas substancias incrementaban el crecimiento de las plantas. El incremento de concentraciones de vanadio causó el incremento en el contenido de clorofila en los pesos secos y frescos de las plantas. Por otro lado, tanto los tratamientos de vanadio como con abono animal, tuvieron similar efecto en la soya.

Palabras clave: Clorofila; soya; vanadio.

Introduction

Vanadium (V) is a trace element that is essential for certain plants and microorganisms (1, 2). Recent studies have shown that vanadium has a positive influence on plants, particularly on legumes. It favors the fixation of nitrogen by nodules and also plays a part in reducing nitrates in some species. Vanadium has an effect that is comparable to that of molybdenum (3). It has been reported that small amounts of vanadium in anion form are stimulating to

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plants, while large amounts are toxic (4). Ten to 20 mg kg^{-1} V in nutrient solution is commonly harmful to plants, but larger amounts can be tolerated by legumes.

The V contents of soils vary from traces to 300 mg kg^{-1}, the average is about 90 to 100 mg kg^{-1} (3; 5). Vanadium can be retained either by the clay fraction or by secondary iron oxides (6). Vanadium also forms a complex with soil organic matter that is able to reduce V (+5) to V (+4) (7- 9).

Phosphorus fertilizers increase soil V levels, and this may cause problems in like market gardening where heavy P dressings are used (10). On the other hand, field studies showed that the presence of V may interfere with the plant's uptake of P even when soils are moderately rich in P (11).

Regarding to soil properties, although no clear correlation has been found between soil pH and V uptake plants on lime-rich soils have a low uptake (10).

Recent V studies were mainly concentrated on sand cultures or hydroponic systems (1, 2, 12, 13). Direct studies related to soil and farmyard manure have been rarely performed.

The aim of this study was to investigate whether V is toxic or not to soybean plants and the effect of V and both V and farmyard manure (FYM) on the growth, mineral and chlorophyll content of soybean plants.

Materials and Methods

Soybean (*Glycine max* (L.) Merr) plants (Corsoy variety) were grown in Kick-Brown pots filled with 8 liter clay loam textured soil, treated with composted farmyard manure at the rate of 1 ton ha^{-1} and 2 ton ha^{-1}. Selected soil properties are given in Table 1. Plants were grown in a greenhouse under natural light (14). Seeds were sown at a rate of three per pot and thinned to one after emergence.

A basal dose of N, P and K was mixed thoroughly into the soil at the 50, 60 and 75 mg kg^{-1}, respectively, before sowing.

Vanadium was applied to the soil samples at the rates of 0, 0.5, 1.0, and 2.0 $\mu g\, g^{-1}$ as a solution of sodium vanadate (Na_3VO_4. $2H_2O$). Soybean seeds were inoculated with *Bradyrhizobium japonicum* USDA 110 strain (15). Plants were irrigated daily to the field capacity level and harvested after maturity.

After weighing the fresh material, plants were washed and 250 mg of fresh material were sampled for chlorophyll analysis. Then plant materials were dried at 70°C in order to determine dry matter, ground and samples were analyzed for total-N, Na, P and K.

Total-N was determined by Kjeldahl method (16). Sodium and K were analyzed in concentrated nitric-perchloric acid (4:1) sample digests by using flame photometry (Jenway PFP 7 model). Phosphorus determination was performed by vanadate-molybdate method (17).

Chlorophyll content was estimated according to the procedure of Withan *et al.* (18). Leaves, 250 mg, were triturated in a porcelain mortar with 80% acetone and filtrated. The filtrate was made up to 50 mL, thoroughly mixed and used to determine total chlorophyll at 652 nm spectrophotometrically (Shimadzu UV-1201 model).

Table 1
Selected soil properties

Org. Matter (%)	pH (1:2.5) soil:water	EC (mmhos cm^{-1})	$CaCO_3$ (%)
1. 85	7. 84	0.172	5.12

Four treatments with three replications were compared in a completely randomised factorial design. The data obtained from the different analyses were evaluated statistically using MSTAT computer program.

Results and Discussion

Effects on plant growth

Phenologic observations showed that V concentrations had no injurious effect on the growing plants. It has been proposed that phytotoxicity of V results in chlorosis and dwarfing may appear at about 2 $\mu g\ V\ g^{-1}$ in some plants (19; 20). Kaplan *et al.* (2) stated that soybeans grown in 3 and 6 mg V L^{-1} treated Hoagland's solution exhibited visual toxicity symptoms. In this study, from beginning of the treatment applications to the harvest there were no toxicity symptoms like darkening, chlorosis or reddening as reported by Kaplan *et al.* (2). This is attributed to the fact that V would not be toxic under field conditions because of its low concentration in the soil solution (21).

Fresh and dry weights of plants indicate that growth was inhibited as V additions increased ($P < 0.01$; Table 2). This effect of V was more pronounced for plants grown at the greater V concentrations. In the absence of applied V, farmyard manure treatments did not also show an effect on the plant growth. On the other hand, both V and farmyard manure treatments caused to increase in both fresh and dry weights of plants. Because soil organic matter is able to reduce V (+5) to V (+4), (7-9), the availability of V to the plants may possibly increase with the reduction of V (+5) to V (+4) by farmyard manure as an organic matter source. It is concluded that plant roots reduce V (+5) to V (+4) to make the element available (12).

Nodule number was unaffected by both treatments except 1 mg kg^{-1} V and 1 ton ha^{-1} FYM doses. This finding can be explained by the presence of nitrogen as a basal fertilizer. When enough nitrogen exists in the soil, developments of nodules and symbiotic N fixation is discouraged. Nodulation also depends on the genetic characters and such environmental effects as soil pH and physical and nutritional factors (22).

Mineral constituents of the plants

The V concentrations in the roots averaged 68 $\mu g\ g^{-1}$ (data not shown). However, the V concentrations in the aerial parts of the plants were below the detection limit (0.02 $\mu g\ g^{-1}$). These observations are in general agreement with those of Warington (23), Notton and Hewitt (24) and Kaplan (2) that a large percentage of the V taken up by plants remains in the roots.

Nitrogen contents of the plants were increased for all treatments as compared to the control plants ($P < 0.01$; Table 3). This increase was greatest for plants grown at 1 mg kg^{-1} V applications. Kaplan *et al.* (2) stated that N contents of soybean plants grown in hydroponic system were increased at 3 mg V L^{-1} treatments beyond which N concentrations decreased with increasing V treatments. It has been reported by Singh (1) that in maize (*Zea mays* L.) plants grown in a sand culture, only 0.05 mg kg^{-1} concentration of vanadium increased N, P and K contents of maize and increasing doses (up to 6.25 mg kg^{-1}) decreased the mineral content.

Phosphorus contents of the plants were unaffected by the vanadium treatments (Table 3). Only 0.5 mg kg^{-1} concentration of vanadium increased P content of plants and beyond this level P content decreased with corresponding increase in vanadium concentrations. This effect of V on the P content supports the findings of Kaplan *et al.* (2).

Potassium content of plants was unaffected by V and FYM treatments ($P > 0.05$; Table 3).

Table 2
Fresh and dry weights of soybean and nodule numbers as affected by vanadium and farmyard manure treatments

V treatments, mg kg^{-1}	Fresh weights, g pot^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	4.43 A•a••	3.54 Bb	3.82 Bb	3.93
0.5	3.87 Bb	3.00 Cc	4.65 Aa	3.84
1	3.03 Cc	4.51 Aa	3.68 Bb	3.74
2	2.12 Bd	4.41 Aa	4.37 Aa	3.63
Average	3.36	3.87	4.13	
V treatments, mg kg^{-1}	Dry weights, g pot^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	1.22 Aa	1.00 Bc	1.14 Ab	1.12
0.5	0.96 Bc	0.86 Bd	1.25 Aa	1.02
1	1.02 Bb	1.20 Aa	1.04 Bc	1.08
2	0.59 Bd	1.09 Ab	1.18 Ab	0.95
Average	0.95	1.04	1.15	
V treatments, mg kg^{-1}	Nodules, number plant^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	6.00 Aa	5.00 Ab	9.00 Aa	6.67
0.5	8.66 Aa	2.00 Bc	8.00 Aa	6.22
1	5.33 Bb	16.66 Aa	6.66 Ba	9.55
2	10.66 Aa	10.00 Ab	7.33 Aa	9.33
Average	7.66	8.42	7.75	

Sources	df	Significance for fresh weight	Significance for dry weight
V	3	**	**
FYM	2	**	**
Vx FYM	6	*	*

*,**: Significant at the 0.05 and 0.01 probability levels, respectively. NS: Not significant.
•: Capital letters for the comparison of FYM treatments.
••: Small letters for the comparison of V treatments.
For fresh weight LSD 0.05: 0.37.
For dry weight LSD:0.05: 0.039.
For nodule numbers LSD: 0.05: 5.19.

Table 3
Nitrogen, P, K contents of soybean plants as affected by vanadium and farmyard manure treatments

V treatments, mg kg^{-1}	N, %			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	4.17 B•c••	4.43 Ab	4.33 Abb	4.31
0.5	4.69 Ab	4.46 Bb	4.50 Ba	4.55
1	5.02 Aa	4.89 Aa	4.51 Aa	4.81
2	4.49 Bb	4.85 Aa	4.56 Ba	4.63
Average	4.59	4.66	4.48	
V treatments, mg kg^{-1}	P, %			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	0.020 Ba	0.028 Aa	0.022 Ba	0.023
0.5	0.025 Ba	0.020 Cb	0.030 Aa	0.025
1	0.021 Ba	0.028 Aa	0.029 Aa	0.026
2	0.016 Cb	0.031 Aa	0.027 Ba	0.025
Average	0.021	0.027	0.027	
V treatments, mg kg^{-1}	K, %			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	0.49	0.55	0.52	0.52
0.5	0.44	0.64	0.62	0.57
1	0.52	0.52	0.58	0.54
2	0.50	0.61	0.61	0.57
Average	0.49 (NS)	0.58 (NS)	0.58 (NS)	

Sources	df	Significance for fresh weight	Significance for dry weight
V	3	**	**
FYM	2	**	**
Vx FYM	6	*	*

*,**: Significant at the 0.05 and 0.01 probability levels, respectively. NS: Not significant.
••: Small letters for the comparison of V treatments.
•: Capital letters for the comparison of FYM treatments.
For N content LSD 0.05: 0.203.
For P content LSD: 0.05: 0.0064.

Table 4
Total chlorophyll content of soybean as affected by vanadium and farmyard manure treatments

V treatments, mg kg^{-1}	Chlorophyll mg g^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	830 B*b**	917 Bc	1440 Ab	1060
0.5	1320 Ba	1040 Bb	1850 Aa	1400
1	1460 Ba	1320 Bb	2060 Aa	1610
2	1450 Aa	1800 Aa	1640 Ab	1630
Average	1270	1270	1750	

Sources	df	Significance for fresh yield weight	Significance for dry yield weight
V	3	**	**
FYM	2	**	**
Vx FYM	6	*	*

*,**: Significant at the 0.05 and 0.01 probability levels, respectively. NS: Not significant.
**: Small letters for the comparison of V treatments.
*: Capital letters for the comparison of FYM treatments.
For Chlorophyll content LSD: 0.05: 363.

Total chlorophyll content of the plants

Chlorophyll content was increased by increasing doses ($P < 0.01$), but the difference was not well marked (Table 4). These results are similar to the sand culture findings of Singh (1). High levels of V treatments (1 and 2 mg kg^{-1}) with FYM manure also increased total chlorophyll contents of the plants.

Fresh and dry yield weights of soybean

Fresh and dry weights and seed yields were unaffected by FYM doses (Table 5). A long-term study on FYM had shown that about 1 ton ha^{-1} FYM applications may be more productive on the plant yields (25). Vanadium with FYM treatments has an effect on fresh and dry yield weight of soybean plants as compared to the control plants ($P < 0.01$). The increase in soybean yield could be related to the in greater dry matter accumulation due to the increase in N uptake. Zade *et al.* (13) reported a similar result in wheat with the foliar application of 50 mg kg^{-1} V.

Conclusion

In general, V without FYM decreased plant weights. With FYM, V had positive effects on vegetative growth of Corsoy variety of soybean. Rates of V applied in the experiment (0.5, 1 and 2 μg g^{-1}) did not show any toxicity to the growing plant. V accumulated in the soybean roots but was not detected in aerial portions of the plants.

Application of FYM with V showed varying effects on soybean growth and its nutrient content depending on their application rates. But in some cases, because of FYM's organic character, effects of the V concentrations on soybean were masked by FYM applications.

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Table 5
Fresh and dry yield weights of soybean and pod numbers as affected by vanadium and farmyard manure treatments

V treatments, mg kg^{-1}	Fresh yield weights, g pot^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	2.71 A•b••	1.91 Cc	2.31 Bc	2.31
0.5	3.47 Ba	2.00 Cc	3.64 Aa	3.04
1	2.95 Bb	2.70 Cb	3.20 Ab	2.95
2	2.25 Cc	3.18 Ba	3.35 Aa	2.93
Average	2.85	2.45	3.13	

V treatments, mg kg^{-1}	Dry yield weights, g pot^{-1}			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	1.54 Ab	1.21 Bc	1.49 Ab	1.41
0.5	2.03 Aa	1.21 Cc	1.86 Ba	1.70
1	1.65 Bb	1.78 Aa	1.65 Bb	1.69
2	1.64 Ab	1.62 Bb	1.47 Cb	1.58
Average	1.72	1.46	1.62	1.60

V treatments, mg kg^{-1}	Pod Numbers			
	Farmyard manure treatments, ton ha^{-1}			
	0	1	2	Average
0	6.00 Aa	4.67 Aa	5.00 Aa	5.22
0.5	7.67 Aa	4.00 Ba	6.67 Aa	6.11
1	5.00 Ab	5.33 Aa	6.00 Aa	5.44
2	5.00 Ab	4.67 Aa	3.33 Bb	4.33
Average	5.92	4.67	5.25	

Sources	df	Significance for fresh weight	Significance for dry weight
V	3	**	**
FYM	2	**	**
Vx FYM	6	*	*

*,**: Significant at the 0.05 and 0.01 probability levels, respectively. NS: Not significant.
•: Capital letters for the comparison of FYM treatments.
••: Small letters for the comparison of V treatments.
For fresh weight LSD: 0.05: 0.31.
For dry weight LSD: 0.05: 0.18.
For nodule numbers LSD: 0.05: 1.88.

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