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Original Article

Effect of vanadium on plant growth and its accumulation in plant tissues

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Abstract

Hydroponic experiments were conducted to investigate vanadium uptake by Chinese green mustard and tomato plants and its effect on their growth. Twenty-eight (Chinese green mustard) and 79 days (tomato) after germination, the plants were exposed for a further seven days to a solution containing six different concentrations of ammonium metavanadate (0-80 mg/l NH_4VO_3). The vanadium accumulated in the plant tissues were determined by ion-interaction high performance liquid chromatography, with confirmation by magnetic sector ICP-MS.

The results indicated that nutrient solution containing more than 40 mg/l NH_4VO_3 affected plant growth for both Chinese green mustard and tomato plant. Chinese green mustard grown in the solution containing NH_4VO_3 at the concentrations of 40 and 80 mg/l had stem length, number of leaves, dry weight of leaf, stem and root significantly lower than those of plants grown in the solution containing 0-20 mg/l NH_4VO_3. Tomato plants were observed to wilt after four days in contact with the nutrient solutions containing 40 and 80 mg/l NH_4VO_3. As the vanadium concentrations increased, a resultant decrease in the stem length, root fresh weight, and fruit fresh weight were noted. The accumulation of vanadium was higher in the root compared with leaf, stem, or fruit. Measured levels of vanadium, from a nutrient solution containing 40 mg/l NH_4VO_3, were 328, 340, and 9.66×10^3 µg/g in the leaf, stem and root for Chinese green mustard, and 4.04 and 4.01×10^3 µg/g in the fruit and roots for tomato plants, respectively.

Key words: HPLC, hydroponics, plant tissue, sample preparation, vanadium

1. Introduction

Vanadium (V) is widely dispersed in the environment in several ways including the leaching of rocks, the combustion of coal or petroleum products, the contamination from the use of fertilizers, and residual slags from the steel industry. As a result, V has been increasingly released into the soil, water and atmosphere (Ringelband and Hehl, 2000).

A previous report (Heinemann *et al*., 2000) showed that patients who had used albumin solutions containing high levels of V could suffer renal damage, especially those patients with existing impaired renal function. Some people

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in communities in northeastern Thailand are known to suffer health problems with regard to distal renal tubular acidosis. This was thought to have arisen from high environmental levels of V in the soil as higher V levels were also found in these patients urine (Tosukhowong *et al*., 1999). V contamination from basic slag fertilizers was observed to have toxic effects on cattle after feeding on contaminated fresh hay (Frank *et al*., 1996). The effect of V on plant growth has also been reported in soybean. It was found that if the concentration of V added to the fluvo-aquic soil exceeded 30 mg/kg soil, significantly decreased yields of shoots and roots were obtained. Also, seedling leaves were yellow and withered (Wang *et al*., 1999). The most toxic oxidation state of V is +5 (vanadate). There is a wealth of evidence that vanadate is absorbed by plant tissues (Bowman, 1983; Ullrich-Eberius *et al*., 1989) and can inhibit the plasma membrane hydrogen (H^+)-translocation ATPase (Vara and Serrano, 1982), which is known to play an important role in nutrient element uptake by plant cells.

Vachirapatama *et al.* (2002) revealed that some phosphate fertilizers were contaminated with high concentrations of V (90-180 mg/kg). This may suggest that the use of these phosphate fertilizers may cause the V to become widely spread in soils, water and vegetables and it has potential effects to human and animal health. In order to obtain more information about the level of V in plant tissues and its effect on plant growth, Chinese green mustard and tomato plants were grown in hydroponic systems containing varying concentrations of V. Therefore, the aims of the present research were to investigate the effect of V on plant growth and to determine the quantity of V in plant tissues using an on-line preconcentration and sample clean up RP-HPLC method for the determination of V as the ternary complex of 4-(2-pyridylazo) resorcinol (PAR) and hydrogen peroxide (Vachirapatama *et al*., 2005).

2. Materials and Methods

2.1 Plant material and growth conditions

Chinese green mustard (*B. campestris ssp. chinensis var. parachinensis*) was cultured hydroponically in a greenhouse at Thammasat University, Thailand. Seeds were grown into sponge cubes (size 3x3 cm) in water for three days and seedlings were then cultured in a half-strength Knop's solution for a week. Plants were transferred to a closed container (31x46.5x18 cm) and placed in holes spaced 2.5 cm apart for 21 days. The containers were filled with 20 liters of full strength Knop's solution containing: 0.20 g/l KNO_3; 0.80 g/l $(CaNO_3)_2.4H_2O$; 0.20 g/l KH_2PO_4; 0.20 g/l $MgSO_4.7H_2O$; 20 mg/l $FeSO_4.7H_2O$; 2.0 mg/l $MnSO_4.4H_2O$; 0.20 mg/l $CuSO_4.5H_2O$; 0.45 mg/l $ZnSO_4.7H_2O$; 2.9 mg/l H_3BO_3; and 0.05 mg/l $Na_2MoO_4.2H_2O$. Solutions were aerated with compressed air that was forced through air-stones placed on the bottom of the container. Plants (28 days after germination) were allowed to grow for a week in Knop's solution containing six different ammonium metavanadate (NH_4VO_3) concentrations: 0, 1, 10, 20, 40, and 80 mg/l. The V solutions were maintained at a constant volume during the experiment. At harvest time, the stem and root length as well as the number of leaves were recorded. Plants were then separated into leaves, stem and roots, and oven dried at 70°C for 48 hours. Dry weight of each part was recorded. The dried plants were ground and passed through a 70 mesh sieve.

Tomato seeds were grown in the same manner as the Chinese green mustard, except the seeds were grown for five days in water for germination. The full strength hydroponics solution used for the tomato plants contained: 0.59 g/l KNO_3; 0.18 g/l KH_2PO_4; 0.74 g/l $(CaNO_3)_2.4H_2O$; 0.02 g/l Fe-EDTA; 0.17 g/l $MgSO_4.7H_2O$; 5.73 mg/l $MnSO_4.4H_2O$; 0.13 mg/l $CuSO_4.5H_2O$; 1.19 mg/l $ZnSO_4.7H_2O$; 1.27 mg/l H_3BO_3; and 0.12 mg/l $Na_2MoO_4.2H_2O$. At an age of 79 days after germination, the tomato plants were allowed to grow for a week in solutions containing six different concentrations of NH_4VO_3: 0, 1, 10, 20, 40, and 80 mg/l. Stem length and stem weight of fruits from each plant were measured before exposure to the V solutions. At harvest time, the stem length and fresh weight of fruit were recorded. Plants were then separated into leaves, fruits and roots, and oven dried at 70°C for 48 hours. Dry weight of each part was then recorded. The ground dried plant components were passed through a 70 mesh sieve.

2.2 Measurement of vanadium in plant tissue

An on-line preconcentration and sample clean-up chromatographic method (Vachirapatama *et al*., 2005) was used to determine V levels in plant tissues. Comparative measurements were made using a magnetic sector ICP-MS system (Finnigan, Bremen, Germany). Screw-top Savillex® Teflon beakers (Savillex, Minnesota, USA) were used for plant sample digestion prior to analysis.

Standard ammonium metavanadate (NH_4VO_3, 99.99% purity) was obtained from Aldrich (Milwaukee, WI, USA.) and a stock solution of 1.00 g/l NH_4VO_3 was prepared in 1.0 M HNO_3. All water used in the chromatographic system was distilled and deionised using a MilliQ water purification system (Millipore, Bedford, MA, USA). HPLC grade methanol was obtained from Merck (Darmstadt, Germany). All other chemicals used were AR grade unless otherwise specified.

2.3 Statistical analysis

The experiments were conducted using the completely randomized design (CRD) with six treatments and three replications. Analysis of variance (ANOVA) was used to determine the statistical significance of the difference between treatment means in all experiments. ANOVAs were calculated using SPSS version 9.0 for Microsoft Window®. Where a significant difference was found, the Duncan's Multiple Range Test (DMRT) at the 5% level of probability was used to compare individual treatment means.

3. Results and Discussion

3.1 Effect of vanadium on plant growth

In Chinese green mustard, with increasing V concentrations, stem length and the number of leaves were significantly decreased whereas root length behaves similarly but was not significantly different (Table 1, Figure 1). The dry weight of leaf, stem and root grown in solutions containing 40 and 80 mg/l NH_4VO_3 were significantly lower than those grown in lower V concentrations (Figure 2). Under a microscope, the lateral roots of 20-80 mg/l NH_4VO_3 treated plants were stunted (Figure 3). The obstruction of water and mineral absorption may have occurred, which result in lower dry weight of shoot and root. This would indicate that higher concentrations of V (40-80 mg/l NH_4VO_3) have an adverse effect on shoot and root growth of Chinese green mustard. The results from this study were consistent with the conclusion of Vachirapatama and Jirakiattikul (2008) where V was found to retard the shoot and root growth of Chinese green mustard grown under substrate culture especially at the highest concentration of NH_4VO_3 (80 mg/l).

Changes in stem length, and fresh and dry weight of the root and fruit were determined for tomato plants cultured in nutrient solutions containing 0-80 mg/l NH_4VO_3. It was found that tomato plants wilted four days after exposure to nutrient solutions containing 40-80 mg/l NH_4VO_3 (Figure 4). As shown in Table 2, the plants treated with 40 and 80 mg/l NH_4VO_3 had significantly lower stem length increase than those of the ones treated with 1 and 10 mg/l NH_4VO_3. At the lower concentrations of NH_4VO_3 (1-10 mg/l) seemed to enhance stem growth (Table 2). This finding was in agreement with Chongkid *et al.* (2007) who reported that the

Table 1. Stem length, root length and leaf number of Chinese green mustard cultured in nutrient solutions containing 0-80 mg/l NH_4VO_3 for seven days. Average data and standard errors are shown for three replicates.

NH_4VO_3 (mg/l)	Stem length (cm)[1/]	Root length (cm)	Number of leaves
0	26.8 ± 1.0^{d}	30.8 ± 2.4	9.2 ± 0.7^{b}
1	28.4 ± 0.8^{d}	28.6 ± 1.1	8.2 ± 0.6^{b}
10	23.5 ± 0.1^{c}	25.9 ± 3.4	8.7 ± 0.2^{b}
20	22.6 ± 1.1^{c}	24.1 ± 1.1	8.3 ± 0.4^{b}
40	18.0 ± 0.6^{b}	21.3 ± 1.4	5.3 ± 0.7^{a}
80	15.7 ± 0.1^{a}	23.9 ± 2.1	4.2 ± 0.3^{a}
F-test	*	ns	*

[1/] Mean followed by the same letter in a column are not significantly different at the 5% level of probability by DMRT.
* = Significantly different at the 5% level of probability.
ns = Not significantly different at the 5% level of probability.

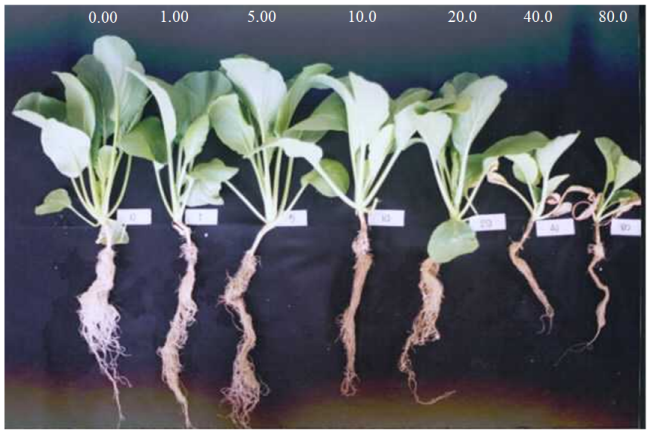

Figure 1. Comparison of chinese green mustard plants growth cultured in nutrient solutions containing 0-80 mg/l NH_4VO_3 for 7 days.

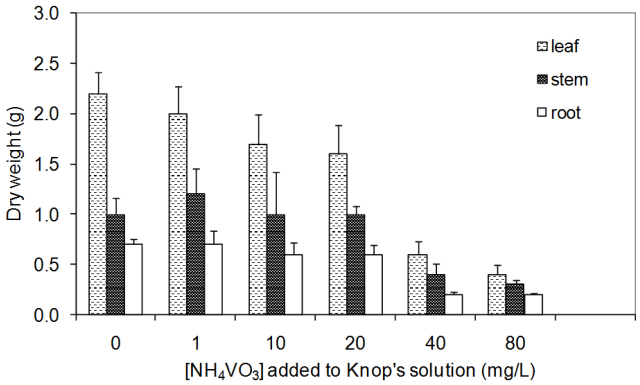

Figure 2. Dry weight of leaf, stem and root of chinese green mustard samples, which were cultured in Knop's solutions containing different ammonium metavanadium concentrations. Data are means of three replicates.

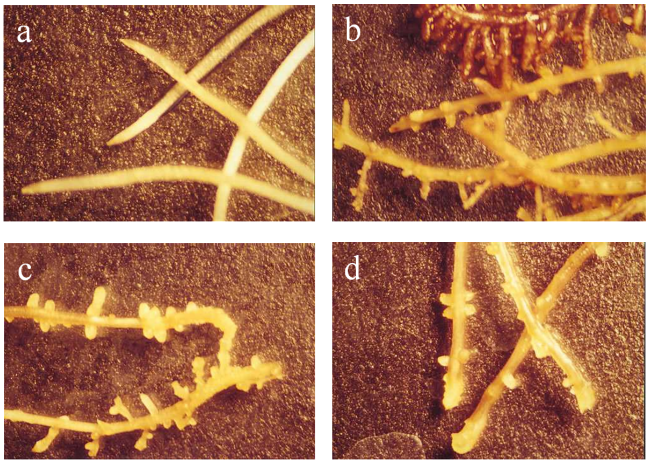

Figure 3. The effect of vanadium on growth of lateral roots. [NH_4VO_3] in Knop's solution = 0.00, 20.0, 40.0 and 80.0 mg/l in a, b, c, and d respectively.

Table 2. Stem length, root and fruit weight of tomato samples after plants were cultured in nutrient solutions containing 0-80 mg/l NH_4VO_3 for seven days. Average data and standard errors are shown for three replicates.

NH_4VO_3 (mg/l)	Stem length increase (cm)[1/]	Root weight (g)		Fruit weight (g)	
		Fresh weight	Dry weight	Fresh weight increase	Dry weight
0	21.3 ± 4.0^{ab}	176.88 ± 8.69^{c}	9.08 ± 0.60	5.08 ± 0.52^{b}	0.74 ± 0.07
1	31.3 ± 4.6^{b}	178.97 ± 19.51^{c}	8.65 ± 0.81	5.23 ± 0.65^{b}	0.75 ± 0.05
10	30.0 ± 0.3^{b}	153.08 ± 4.76^{bc}	7.43 ± 0.31	6.00 ± 0.26^{b}	0.75 ± 0.09
20	20.7 ± 3.5^{ab}	119.29 ± 4.87^{ab}	7.41 ± 0.44	5.68 ± 0.18^{b}	0.78 ± 0.04
40	11.0 ± 1.0^{a}	103.31 ± 7.08^{ab}	7.56 ± 0.76	3.26 ± 0.13^{a}	0.76 ± 0.03
80	9.5 ± 1.7^{a}	96.24 ± 4.68^{a}	7.08 ± 0.56	3.52 ± 0.37^{a}	0.71 ± 0.04
F-test	*	*	ns	*	ns

[1/] Mean followed by the same letter in a column are not significantly different at the 5% level of probability by DMRT.
* = Significantly different at the 5% level of probability.
ns = Not significantly different at the 5% level of probability.

highest fresh and dry stem weight of rice was found when plants were treated with 10 mg/l NH_4VO_3. This effect may be due to the fact that V at this concentration can help increase nitrogen in the form of ammonium compound activating the rice growth. Arnon and Wessel (1953 cited in Welch and Huffman, 1973) also reported that V is an essential element for the growth of the green alga (*Scenedesmus obliquus*) which required 0.1 g/ml V in the nutrient medium. It has been concluded if V is an essential element for lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum*), the adequate tissue level would be less than 2 nanograms per gram V derivable from a growth medium containing less than 0.04 nanogram per milliliter V (Welch and Huffman, 1973).

Vanadium toxicity to tomato roots was also evidenced by a browning of the root structure (Figure 5) when plants were cultured in solutions containing 20-80 mg/l NH_4VO_3. Data also showed that the fresh weight of root significantly decreased with increased concentration of NH_4VO_3 in the nutrient solution (Table 2). This may indicate that V at high concentrations has an effect on root growth even though the dry weight of root was not significantly different among the treatments. Similarly, those treated plants with 40-80 mg/l NH_4VO_3 had the lowest fresh fruit weight increase, while the dry weight of fruit was statistically unaffected (Table 2). This may be because the roots of these plants were damaged and could not function efficiently. Therefore, fruit fresh weight of the treated plants at high V concentrations was lower than those of other treatments. This was consistent with the observation of Chongkid *et al.* (2007) where the lowest growth of rice plant occurred after growing in nutrient solution containing 80 mg/l NH_4VO_3 and 40-80 mg/l NH_4VO_3 caused death to the rice plant before the flowering stage. In addition, Wang and Liu (1999) indicated that yields of soybean shoot and root were significantly decreased in plants receiving V more than 30 mg/kg soil. The inhibitory effect of V has been reported by Vara and Serrano (1982) that vanadate inhibits the plasma membrane hydrogen (H+)-translocating ATPase, which is known to have significant roles in nutrient element uptake by the plant cell. It also inhibits phosphatase (Gallagher and Leonard, 1982) and fructose 2,6-bisphosphatase (Brauer and Stitt, 1990), which are involved in phosphorus and photosynthetic, respectively. In addition,

Figure 4 Comparison between tomato plants grown in nutrient solutions containing ammonium metavanadate at concentrations of 0 mg/l (left) and 80.0 mg/l (right) for 7 days.

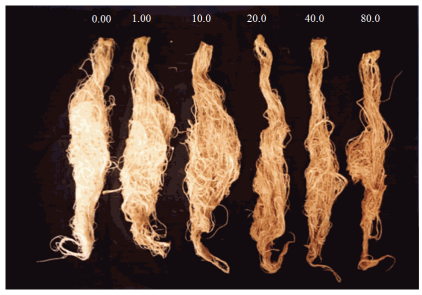

Figure 5. Comparison of tomato roots cultured hydroponically in nutrient solutions treated with ammonium metavanadate at the concentrations of 0, 1, 10, 20, 40 and 80 mg/l for 7 days (left to right).

Wuilloud *et al.* (2000) found that V was a barrier for an activity of plasma membrane hydrogen ion in transferring the ATPase, which is necessary for essential mineral absorption of some plant tissue. Such those effects of V, the retarded growth of Chinese green mustard and tomato plants grown in high concentration of NH_4VO_3 occurred as shown in the results of this study.

3.2 Vanadium concentration in plant tissues

Vanadium levels were analyzed in dry leaf, stem and root of the Chinese green mustard plants and also in the dry root and fruit of the tomato plants that were cultured in nutrient solutions containing 0, 1, 10, 20, 40 and 80 mg/l NH_4VO_3. For validation purposes Chinese green mustard plant digests were analyzed by magnetic sector ICP-MS.

Due to the complex nature of the plant digests, V quantitation by HPLC was performed using the method of standard additions. The results indicated that the amount of V accumulated in the root was higher than in either the stem or the leaf of Chinese green mustard (Table 3). For tomato plants, very high V accumulations were also found in the roots, however lower levels were measured in the fruit (Table 4). Good agreement was found between the V levels in the Chinese green mustard samples by both HPLC and ICP-MS methods, as detailed in Table 3 (R=0.9995, slope=1.02). The results from this study were in agreement with Chongkid *et al.* (2007) who reported that the V concentrations were found in the rice root greater than leafy stem and seed when the rice plants were grown in nutrient solution containing NH_4VO_3.

4. Conclusion

Vanadium was found to retard the growth of Chinese green mustard and tomato plants, especially for nutrient solutions containing more than 40 mg/l NH_4VO_3. It was also found that V accumulated at higher levels in root compare to stem and leaf in Chinese green mustard samples. For tomato plants, V accumulated at higher levels in root compared to the fruit.

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Table 3. Comparative results of [V] found in Chinese green mustard samples by HPLC and magnetic sector ICP-MS. Average data are shown for two replicates.

NH_4VO_3 (mg/l)	Chinese green mustard samples	[V] found (mg/kg in solid samples)	
		HPLC	ICP-MS
0	Leaf	4.73	3.65
	Stem	2.06	2.30
	Root	58.4	62.7
1	Leaf	16.7	15.2
	Stem	25.7	26.4
	Root	1.95×10^3	2.05×10^3
10	Leaf	50.9	52.1
	Stem	59.9	63.7
	Root	6.96×10^3	6.50×10^3
20	Leaf	140	141
	Stem	141	132
	Root	9.00×10^3	8.79×10^3
40	Leaf	328	335
	Stem	340	350
	Root	9.66×10^3	9.57×10^3
80	Leaf	477	504
	Stem	823	791
	Root	1.35×10^4	1.34×10^4

Table 4. Vanadium concentrations found in tomato samples (fruit and root) by HPLC. Average data are shown for two replicates.

NH_4VO_3 (mg/l)	Tomato samples (type)	[V] found (mg/kg in dry samples)
0	Fruit	0.210
	Root	4.92
1	Fruit	0.49
	Root	273
10	Fruit	0.460
	Root	$1.31x10^3$
20	Fruit	2.86
	Root	$2.46x10^3$
40	Fruit	4.04
	Root	$4.01x10^3$
80	Fruit	8.15
	Root	$5.24x10^3$

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