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Original Article

Effect of vanadium on growth of Chinese green mustard (*Brassica campestris* ssp. *chinensis* var. *parachinensis*) under substrate culture

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Abstract

The effects of vanadium on growth of Chinese green mustard (*Brassica campestris* spp. var. *parachinensis*) and its accumulation in plant tissues were investigated. Substrate culture was set to grow the plants. Two weeks after planting, plants were exposed for 3 weeks with a solution containing 6 different concentrations of ammonium metavanadate (0-80 mg/l). The results showed that stem length, number of leaves, and leaf and stem fresh weight of the plants treated with solution containing 80 mg/l ammonium metavanadate were significantly lower than those of the plants treated with solutions of 0-40 mg/l ammonium metavanadate. The vanadium accumulated in the plant tissues and substrates were determined by ion-interaction high performance liquid chromatography technique. Higher concentration of ammonium metavanadate applied on plant, resulted on higher vanadium accumulated in tissues. Substrate, leaf and stem tissues of plants treated with 80 mg/l ammonium metavanadate showed the highest accumulation of vanadium at 20.981, 1.658 and 2.046 mg/g, respectively.

Key words: *Brassica campestris*, HPLC, hydroponics, substrate culture, vanadium

1. Introduction

Vanadium (V) in the environment comes from different sources such as the leaching of natural rock, the contaminated from usage 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). Vachirapatama *et al*. (2002) showed that phosphate rocks and fertilizers were contaminated with high concentrations of V (90-180 mg/kg). This may suggest that use of these phosphate rocks and phosphate fertilizers may cause the V to become widely spread in soil, water and vegetables. From survey of surface soil in the US, it was found that about 14% of all sites contained more than 120 mg/g of V and about 60% of all sites contained more than 38 mg/g of V (Shacklette *et al*., 1971). Vanadium has potential effects on human and animal health and on plant growth. 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 communities in north eastern 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 (Tosukhowong *et al*., 1999). Vanadium contamination from basic slag fertilizers was also observed to have toxic effects on mammals. Many cattle were reported to have been poisoned after feeding on contaminated fresh hay (Frank *et al*., 1996). In addition, V inhibits young spring barley growth (Davis *et al*., 1978). The effect of V on plant growth has also been reported in soybean. It was found that the concentration of V added to the fluvo-aquic soil exceeded 30 mg/kg, significantly decreased yields of shoots and roots were obtained. Also, seedling leaves were yellow and withered (Wang and

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Liu, 1999). Hydroponics culture was used to evaluate the relative effect of V on cuphea growth. The result showed that relative root length, root surface area, root weight and aerial dry weight decreased as the V concentration increased from 0 to 153 mM. Furthermore, increases in V concentration significantly reduced secondary and higher order lateral branching of cuphea root (Olness *et al*., 2005). Vanadium has +2, +3, +4 and +5 oxidation state but the most toxic oxidation state 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^+)-translocating ATPase (Vara and Serrano, 1982), which is known to play important roles in nutrient element uptake by plant cells.

Because of the effect of V on plant growth and human and animal health, more information on the level of V in plant tissues, which are consumed by mammals, and its effect on plant growth, is important. Therefore, the aims of the present research were to investigate the effect of V on Chinese green mustard growth under substrate culture technique. On-line preconcentration and sample clean up ion-interaction 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) was used to determine the accumulation of V in substrate, leaf and stem of plant tissues.

2. Materials and Methods

2.1 Plant material, growth conditions and treatments

Chinese green mustard (*Brassica campestris* ssp. *chinensis* var. *parachinensis*) seeds were grown in substrate of sand:carbonized rice hull (1:1, v/v). Seedlings, 4 days after planting, were watered with a half strength Knop's solution for 3 days followed by full strength Knop's solution for a week. Knop's solution is a mixture of essential elements commonly used in hydroponics systems containing: 0.20 g/l KNO_3; 0.80 g/l Ca $(NO_3)_2.4H_2O$; 0.20 g/l KH_2PO_4; 0.20 g/l $MgSO_4.7H_2O$; 26.4 mg/l Fe-EDTA; 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 $Na_2MoO_4.2H_2O$. Nutrient solution was adjusted to pH 5.7 and electrical conductivity of nutrient solution was 1.4 mS/cm. Two weeks after planting, the seedlings were treated with Knop's solution containing 6 different concentrations of ammonium metavanadate (NH_4VO_3): 0, 1, 10, 20, 40 and 80 mg/l for 3 weeks. The experimental structure was based on a Completely Randomised Design (CRD) having 3 replicates and 3 plants per replicate. Plant height and number of leaves were recorded every week after treated with NH_4VO_3. Leaves and stem were separated at harvest, fresh weight were recorded and dried at 70°C for 48 hours. The dried plant tissues were ground and passed through a 70 mesh sieve.

2.2 Sample digestion

Powder of plant samples were digested by using 2 ml of concentrated HNO_3 and 1 ml of concentrated HCl. Each solution was heated on a hotplate at 80°C until clear solution obtained. The solutions were then transferred to a 25 ml volumetric flask and made up to volume with water before final filtration through a 0.45 μm filter.

2.3 Vanadium complex formation and chromatographic method

The experimental methodology used for preparation of the V as a ternary complex with 4-(2-pyridylazo) resorcinol (PAR) and hydrogen peroxide (H_2O_2), and the optimum ion-interaction RP-HPLC conditions were almost the same as those described elsewhere (Vachirapatama *et al*., 2005). The differences were the length of the preconcentration time was 1.60 min and the optimized time for the V complex to be eluted from the guard column on to the analytical column was 0.65 min.

The ternary V complex was prepared by adding 2 ml digested solution to acetate buffer, followed by PAR and H_2O_2, respectively. The solution was then adjusted to pH 6 before diluted to 25 ml with water. The final concentrations of PAR, acetate and H_2O_2 in solution were 0.30 mM, 10 mM and 0.71 mM, respectively.

Mobile phase for sample clean up at guard column was eluent 1 (20% v/v methanol containing 5 mM acetic acid, 10 mM tetrabutylammonium bromide and 5 mM citric acid at pH 7) and for analytical column was eluent 2 (32% v/v methanol containing 5 mM acetic acid, 3 mM tetrabutylammonium bromide and 5 mM citric acid at pH 7). Guard column of NovaPak C_{18} (4 mm) was used as a concentrator column and NovaPak C18 (150 mmx3.9 mm i.d., particle size 4 mm) was used as an analytical column. The temperature was kept at 30°C and detection wavelength was 540 nm.

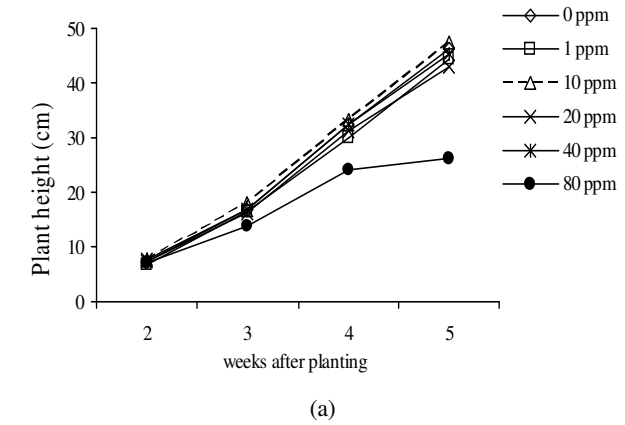
2.4 Statistical analysis

Analysis of variance (ANOVA) was used to determine the statistical significance of the difference between treatment means in all experiments. ANOVAs were calculated using SAS program. Where a significant difference was found, the Duncan's New Multiple Range Test (DMRT) at the 5% level of probability was used to compare individual treatment means.

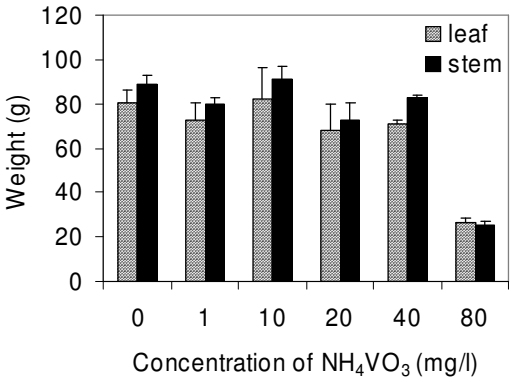
3. Results and Discussion

3.1 Effect of vanadium on Chinese green mustard growth

The growth of Chinese green mustard was not significantly different among the treatments at the first week after



(a)



(b)

Figure 1. Plant height (a) and number of leaves (b) of the Chinese green mustard treated with nutrient solution containing 0-80 mg/l NH_4VO_3 at the 2nd, 3rd, 4th and 5th week after planting.

Figure 2. Leaves of Chinese green mustard plants were withered after treated with nutrient solution containing 80 mg/l NH_4VO_3 for 2 weeks.

treated with NH_4VO_3. However, the plant height and the number of leaves of the Chinese green mustard treated with 80 mg/l NH_4VO_3 were significantly lower than those of the 0-40 mg/l NH_4VO_3 treated plants during the last two weeks (Figure 1). Figure 2 shows withered leaves of some plants after treatment with 80 mg/l NH_4VO_3 for 2 weeks. There was no symptom of V toxicity on the plants treated with 0-40 mg/l NH_4VO_3. At the harvest time, the plants treated with the concentration of 80 mg/l NH_4VO_3 had the lowest of leaf and stem fresh weight of 26.81 g and 25.60 g, respectively (Figure 3). The results also showed that root growth was strongly affected by V at the highest concentration. Under a microscope, the lateral roots of 80 mg/l NH_4VO_3 treated plants were stunted (Figure 4). It can be concluded that the highest concentration of NH_4VO_3 (80 mg/l) had an adverse effect on both shoot and root growth when plants were grown under substrate culture. The results from this study are in agreement with the observation of Wang and Liu (1999) where significantly decreased yield of soybean shoots and roots were obtained in plants receiving V more than 30 mg/kg soil. The inhibitory effect of V has been noted by Vara and Serrano (1982) who found 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

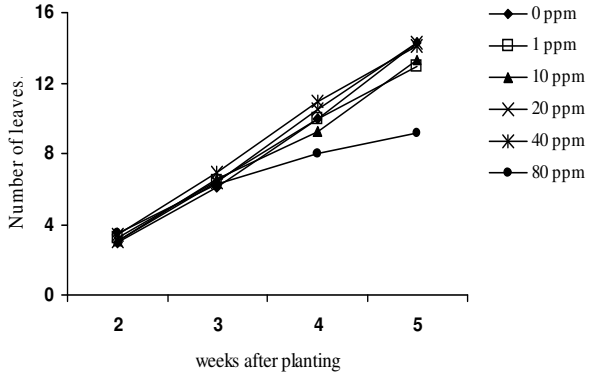
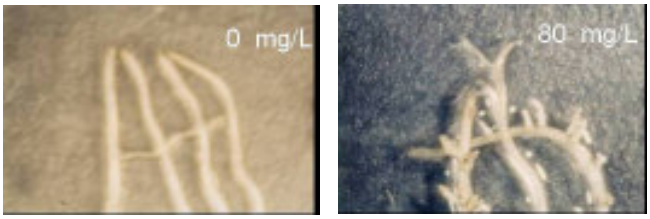


Figure 3. Fresh weight of leaf and stem of the Chinese green mustard treated with nutrient solution containing 0-80 mg/l NH_4VO_3. Vertical bars represent standard deviation (SD) of the means.

(a)



(b)

Figure 4. (a) Root of Chinese green mustard after treated with nutrient solution containing 0 mg/l (left) and 80 mg/l (right) NH_4VO_3 at the harvest time, (b) magnificent x 6.7.

Table 1. Vanadium concentration found in the substrate and the plant tissue of Chinese green mustard by HPLC. Average data are shown for 3 replicates.

NH_4VO_3 concentration (mg/l)	[v] found in substrate[1/] (mg/g)	[v] found in plant tissues (mg/g)[1/]	
		leaf	stem
0	0.224[d]	0.028[b]	0.016[b]
1	1.453[d]	0.093[b]	0.026[b]
10	3.312[d]	0.164[b]	0.150[b]
20	7.669[c]	0.363[b]	0.233[b]
40	12.212[b]	0.479[b]	0.404[b]
80	20.981[a]	1.658[a]	2.046[a]

[1/] Means followed by the same letter in the column are not significantly different at P>0.05.

Stitt, 1990). However, Arnon and Wessel (1953 cited in Welch and Huffman, 1973) 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 also been suggested that V is an essential element for lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum*) when a growth medium contained V less than 0.04 ng/ml (Welch and Huffman, 1973).

3.2 Determination of vanadium in plant tissues

The V uptake by Chinese green mustard plant tissues cultured in substrate treated with nutrient solution containing 0, 1, 10, 20, 40 and 80 mg/l NH_4VO_3 is shown in Table 1. The source of the V contamination in the control treatment came from the reagents and water used to prepare solution, whilst contamination of V in substrate material came from sand and carbonized rice hull. Figure 5 shows the typical chromatogram obtained from control (a); Chinese green mustard stem treated with nutrient solution containing 10 mg/l NH_4VO_3 (b); and solution from (b) spiked with 2 ng/mL V (c). These diagrams indicated that the V complex was well resolved from other components present in the stem samples, with the spiked sample being used to confirm the identity of the peak due to the V complex. Analyses of leaf and substrate samples showed similar chromatograms. Due to the complex nature of the plant digests, V quantitation by HPLC was performed using the method of standard additions. The results from Table 1 indicate that the amount of V accumulated in the substrate was significantly higher than in either the stem or the leaf of Chinese green mustard. In addition, the higher concentrations of NH_4VO_3 treated to substrates and plants provided the higher concentrations of V accumulated in substrates and plant tissues. The results from this study were similar to Chongkid *et al*. (2007) works where the higher the concentration in the nutrient solution, the more V found in the leafy stem and the root of rice. The results also indicated that the V accumulated in rice tissues at higher levels in the root than in the leafy stem and in the seeds.

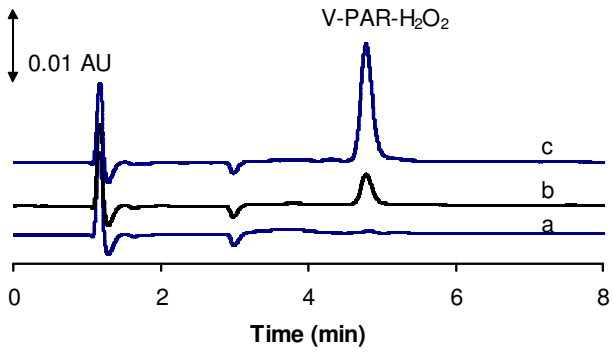


Figure 5. Chromatogram of (a) control; (b) Chinese green mustrad stems culture in substrate treated with nutrient solution containing 10 mg/l NH_4VO_3; (c) same as (b) except spiked with 2 ng/mL vanadium.

4. Conclusions

Vanadium was found to retard the growth of Chinese green mustard plants especially for nutrient solution containing 80 mg/l NH_4VO_3. Results suggested that the lowest plant yield was obtained using the nutrient solution containing 80 mg/l NH_4VO_3. Concentrations of V in substrates and plant tissues were successfully determined by ion interaction high performance liquid chromatography. V accumulated at higher levels in substrates compared to stems and leaves in Chinese green mustard samples.

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