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Vanadium distribution in roots and leaves of *Phaseolus vulgaris*: morphological and ultrastructural effects

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Abstract

In different plant species, vanadium has been considered either as beneficial or as a toxic element, or even as secondary metabolism elicitor, but the mechanisms involved are still not completely understood. In this study, the responses of *Phaseolus vulgaris* L. cv. Contender roots and leaves to different vanadyl sulfate concentrations were studied. The plants grown hydroponically with V had thicker roots, a less developed main root, and a smaller number of secondary roots than the control plants. The V content in roots and leaves was correlated with V supply concentration but the V content in leaf was always much lower than in the root which leads us to conclusion that V accumulates in the roots and only small quantities are transferred to the leaves. However, thylakoid disorganisation was observed in the chloroplasts of plants grown with vanadyl sulphate.

Additional key words: chloroplast ultrastructure, leaf and root growth, TEM.

Introduction

Vanadium is a trace element which is widely distributed in nature (Kaplan *et al*. 1990, Pyrzinska and Wierzbicki 2004) and like other elements (Cu, Cd, Ni, Pb, Cr, Zn) can also be present in contaminated soils (Vwioko *et al*. 2006). Environmental V concentrations started to rise in the 1960s and have increased exponentially in subsequent decades (Peñuelas and Filella 2002). Although V has been identified as an essential micronutrient in green algae, higher concentrations can alter the photosynthesis of freshwater phytoplankton (Nalewajko *et al*. 1995) and cause ultrastructural changes in the chloroplast lamellae of *Chlorella fusca* (Meisch *et al*. 1980). Vanadium is also essential for several species of fungi and nitrogen-fixing microorganisms (Anke 2004), but there is little evidence whether it is essential for higher plants.

Vanadium contamination induced shoot mortality and chlorosis in *Salicornia virginica* plants (Rosso *et al*. 2005) and plants growing in V-contaminated soils were smaller in comparison with the plants growing in areas further away from V mines (Panichev *et al*. 2006). Wheat, rye, and red clover are sensitive to V and can be used as indicators of bioavailable vanadium (Anke 2004). Other species, such as *Cichorium intybus*, *Eupatorium capillifolium* (Martin *et al.* 1996), *Astragalus ssp*., *Allium macropetalum*, *Castillega augustifolia,* and *Chrysothamnus viscidiflorus* (Vwioko *et al*. 2006), have also been studied as potential V indicators.

In *Phaseolus vulgaris* and *Glycine max* grown in hydroponic culture, V altered nutrition, reduced plant biomass and induced visual toxicity symptoms (Kaplan *et al*. 1990, Martín and Saco 1995). Similar biomass reduction has also been described in tomato plants treated with chromium (Henriques 2010). In contrast, vanadyl sulfate has been studied as a possible elicitor of secondary metabolite production for some species (Palazón *et al*. 2003, Khosroushahi *et al*. 2006) and this has increased interest in possible V effects on vascular plants.

The variability in the response to vanadium stress suggests different mechanisms of action (Rosso *et al.* 2005). Some metals tend to accumulate and be retained in the roots (Morrell *et al*. 1986, Gallagher *et al*. 2008), whereas others are mobile and tend to be distributed throughout the plant (Ben Ammar *et al*. 2008). Involved mechanisms are still not completely understood and further investigation is necessary (Shah and Nongkynrih 2007, Gallagher *et al*. 2008).

In this sense, the objective of this study was to

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Abbreviations: DM - dry mass; FM - fresh mass; TEM - transmission electron microscopy.

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investigate the effects of different V concentrations on its uptake and distribution and evaluate several morphological and ultrastructural changes induced by V in leaves and roots of hydroponically grown bean plants.

Materials and methods

Seeds of *Phaseolus vulgaris* L. cv. Contender were germinated on moistened *Vermiculite*. After 7 d, a total of 140 seedlings were transferred to *Vermiculite* with a nutrient solution devoid of vanadium (control plants) or supplemented with increasing concentrations (160, 240, 320, 400 µM) of $VOSO_4 . 5 H_2O$. These concentrations were chosen according to our previous research (Martín and Saco 1989, 1995, Saco *et al.* 1992, 1996). The pH of the culture solution was between 4.5 and 6.0. The plants were watered daily, and the culture solution was not recycled. The environmental conditions were maintained at a 16-h photoperiod with irradiance of 200 µmol m^{-2} s^{-1}, an average temperature of 20 ± 2 ºC, and a relative humidity between 55 - 60 %. Treatments were replicated three times. Plants were harvested at the end of the vegetative stage (30 d), and divided into roots and leaves. The results represent the average of three determinations and the data have been analyzed by Student *t*-test and *ANOVA*.

The parameters used to analyse plant growth were length, width, and surface area of leaves and roots, measured by *Delta-T* image analysis system, and fresh and dry masses of leaves and roots determined after oven-drying to a constant mass at 80 ºC. For determination of V content, root and shoot dry masses were digested in concentrated nitric acid in a *Millestone 1600* microwave oven. V concentration was determined by an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES) according to Chen and Owens (2008). The analysis was carried out in the Atomic Spectrophotometry Centre at the Complutense University in Madrid.

Samples of leaves and roots from control and treated plants were fixed in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0) at 4 ºC for 3 - 4 h, and overnight in the same sodium cacodylate buffer. The samples were then postfixed in 1 % osmium tetroxide in the same buffer at 22 ºC for 1 h. The samples were dehydrated with acetone series, and Spurr's epoxy resin (*SPRLV*, *Sigma-Aldrich*, St. Louis, USA) was used to infiltrate and embed the samples (Liu and Kottke 2004, Villanueva *et al.* 2005, Adamakis *et al.* 2008). Ultrathin sections were cut and stained with uranyl acetate and lead citrate and observed with an transmission electron microscope (*TEM-902*, *Zeiss*, Jena, Germany).

Results and discussion

Duration of the vegetative development stage was similar in control and V-treated plants (30 d). The plants grown with V showed thicker roots, a less developed main root, and a smaller number of secondary roots. As a result, there were significant declines in root length and area (Table 1). This agrees with the results obtained for *Brassica oleracea* plants grown at different V concentrations (Kaplan *et al.* 1990). Similar results were obtained in other species treated with heavy metals (Potters *et al.* 2007),or grown under salinity (Degenhardt and Gimler 2000).

Higher V concentrations (320 and 400 µM) also decreased the root fresh mass (FM) (Table 1). However, there was no overall effect of V on root dry mass (DM) and 240 µM V even caused an increase in root DM. A negative effect of V on root development was detected also in cuphea (Olness *et al.* 2005) but increase in root elongation was observed in *Allium cepa* (Liu and Kottke 2004). According to Tang *et al.* (2009), these facts suggest that certain metals can be stimulatory for the growth of several species at low concentrations but inhibitory at higher concentrations.

Table 1. Effects of different V concentrations in nutrient solution on *Phaseolus vulgaris* root and leaf growth parameters. Means ± SD (*n* = 5); different letters indicate statistically significant differences at $P \leq 0.05$.

Parameters	Control	160 µM V	240 µM V	320 µM V	400 µM V
Root length [cm]	10.32 ± 1.97	9.83 ± 0.29	9.76 ± 0.65	9.47 ± 0.76	5.41 ± 2.98
Root area [cm^2]	16.66 ± 1.67	14.57 ± 1.98	14.18 ± 2.16	13.25 ± 2.26	13.99 ± 2.04
Root FM [g]	0.994 ± 0.13	0.888 ± 0.24	1.046 ± 0.36	0.802 ± 0.32	0.827 ± 0.23
Root DM [g]	0.065 ± 0.01	0.066 ± 0.02	0.080 ± 0.02	0.069 ± 0.02	0.068 ± 0.01
Leaf length [cm]	15.26 ± 4.09	12.47 ± 3.18	12.33 ± 2.87	12.15 ± 2.83	11.70 ± 2.84
Leaf width [cm]	10.58 ± 3.06	8.63 ± 2.33	8.59 ± 2.34	8.63 ± 2.14	8.74 ± 2.39
Leaf FM [g]	5.345 ± 2.24^{b}	4.075 ± 1.69^{ab}	3.455 ± 1.36^{ab}	3.189 ± 1.10^{a}	2.306 ± 0.61^{a}
Leaf DM [g]	0.422 ± 0.17^{d}	0.370 ± 0.08^{cd}	0.324 ± 0.10^{bcd}	0.305 ± 0.10^{abc}	0.189 ± 0.06^{ab}

In addition to significant declines in leaf length, width, and area, significant ($P < 0.01$) negative relationship ($r = -0.993$, $r = -0.944$) were observed between the V supply and the leaf FM and DM. V at concentrations over 240 µM caused shorter, wider, and more rounded leaves with a smaller area. This sharp decrease in the growth of the aerial parts has also been described in plants from other species (Gil *et al.* 1995, Anke 2004, Olness *et al.* 2005, Rosso *et al.* 2005). The decrease in dry mass of the leaves (Table 1) might be due to the decrease in photosynthetic activity, a situation that has also been described for some other heavy metals such as cadmium (Ekmekçi *et al.* 2008, Rascio *et al.* 2008), chromium and copper (Gardea-Torresdey *et al.* 2004).

Table 2. Effects of different V concentration in nutrient solution on root and leaf V content [µg g^{-1}(DM)]. Means ± SD ($n = 5$); different letters indicate statistically significant differences at $P \leq 0.05$.

	Control	160 µM V	240 µM V	320 µM V
Root	16.1 ± 0.8^a	532.0 ± 16^b	1192.0 ± 36^c	1544.0 ± 46^d
Leaf	1.3 ± 0.1^a	8.2 ± 0.4^b	13.6 ± 0.7^c	30.0 ± 2.0^d

Vanadium content in the root was significantly ($P < 0.01$) positively correlated with V concentration of the culture solution ($r = 0.982$; Table 2). Negative relationships were observed between the root V content and root length and area but not FM or DM (Tables 1, 2). In all treatments, V content in the root was always higher than in the nutrient solution and much greater than in the aerial parts of the same plants (around 50 times; Table 2). This has also been described by Morrell *et al.* (1986). The accumulation of metal in the root has also been observed in other species grown in the presence of different heavy metals, *e.g.*, in *Phaseolus vulgaris* grown in substrate with Cd, Ti, and V (Kaplan *et al.* 1990, Martin and Kaplan 1998), seedlings of *Lycopersicum esculentum*, *Vialone nano*, and *Zea mays* grown in the presence of Cd (Ben Ammar *et al.* 2008, Ekmekçi *et al.* 2008, Rascio *et al.* 2008), and tomato plants treated with different forms of chromium (Henriques 2010).

Vanadium content in leaves showed a significant ($0.05 < P < 0.01$) positive correlation ($r = 0.921$) with concentration in nutrient solution (Table 2). The increase in V content in leaves coincided with the decrease in FM and DM (Tables 1, 2). There was also a positive correlation ($r = 0.933$, $0.05 < P < 0.1$) between the leaf and root V content (Table 2). The linear regressions between root and stem or leaf V content were presented by Morrell *et al.* (1986). The much lower V content in leaves than in roots (around 2 %) leads to conclusion that V accumulates in the roots and only small quantities are transferred to the leaves (Gil *et al.* 1995, Ekmekçi *et al.* 2008; Table 2). The ability of *Phaseolus vulgaris* to accumulate V in the roots could be considered as a tolerance mechanism of the species (Wang *et al.* 2008).

In the root, V-treatment caused cell plasmolysis, the alterations in the vacuole morphology were similar to those observed in pea and cotton plants treated with tungsten (Adamakis *et al.* 2008). Increased width of the intercellular spaces was also observed in the cortical parenchyma of V-treated plants (Fig. 1) and this was probably related to V absorption and distribution and to the capacity of roots to accumulate V. Intercellular spaces of different size were also observed in the cortex of *Thlaspi caerulescens* grown in the presence of cadmium and these were suggested to be due to decomposition of middle lamella and distension of cell walls (Wójcik *et al.* 2005).

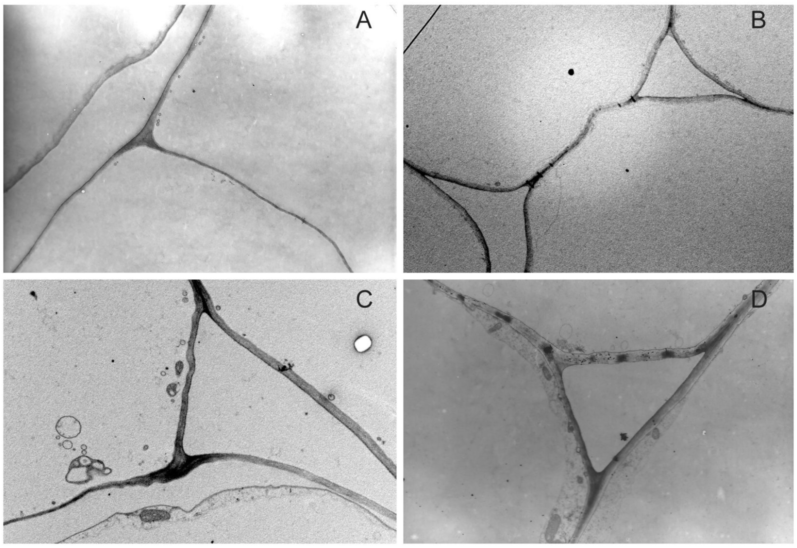

Fig. 1. Transmission electronic micrographs of *Phaseolus vulgaris* root sections. Note the different size of the intercellular spaces between root cortical cells. *A* - control root (4 400×). *B* - root from 160 µM V-treated plant (4 000×), *C* - root from 240 µM V-treated plant (12 000×), *D* - root from 320 µM V-treated plant (4 400×).

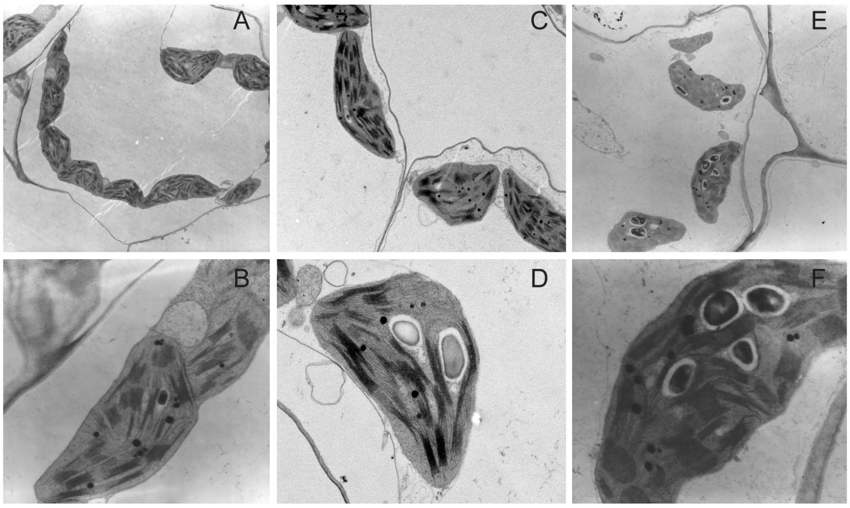

Fig. 2. TEM of *Phaseolus vulgaris* leaf sections. Structural effects of V on leaf mesophyll cells. Control leaf sections: (*A* 3000×), (*B* 12000×); 240 µM V leaf sections: (*C* 3000×), (*D* 12000×); 320 µM V leaf sections: (*E* 3000×), (*F* 12000×).

The ultrastructural studies done with TEM showed significant differences in leaf mesophyll cells of plants receiving the different V concentrations. Fewer chloroplasts were observed in the cells at 240 and 320 µM V than in the leaves of control plants (Fig. 2), similar to observations on cells of *Chlorella fusca* grown in medium with V (Meisch *et al*. 1980).

The chloroplasts of the V-treated plants were larger and contained more starch than those of control plants. The supply of V also led to thylakoid disorganisation which increased with V concentration in the nutrient solution (Fig. 2). The alteration in the membranes of the chloroplasts has also been observed in cells of *Chlorella fusca* (Meisch *et al*. 1980), in rice plants (Rascio *et al*. 2008), and in *Elodea* (Dalla Vecchia *et al*. 2005) grown with Cd.

To summarise our results, V content in both leaves and roots increased with increasing V concentration in the nutrient solution. The leaf V content was always much lower than root V content which leads us to conclude that V accumulates in the root of *P. vulgaris*, and only small quantities are transferred to the leaves. This may be considered as one mechanism of tolerance (Rascio and Navari-Izzo 2011). The morphology of both the leaves (smaller, rounder) and the roots (shorter, lower surface area) changed as V supply increased. Intercellular spaces were modified in the roots probably by the uptake and distribution of the element. The decrease in the number of chloroplasts and the disorganization of thylacoids observed in the leaves suggest possible changes in the photosynthetic activity that might be confirmed in future studies.

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