EFFECTS OF PHOSPHATE, SULFATE AND MOLIBDATE ON THE UPTAKE AND DISTRIBUTION OF TECHNETIUM-99 IN BUSH BEAN PLANTS

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RESUMEN

EFECTOS DEL FOSFATO, SULFATO Y MOLIBDATO SOBRE LA CAPTACION Y DISTRIBUCION DEL TECNECIO-99 EN JUDIA

Se ha estudiado el efecto del fosfato, sulfato y molibdato sobre la captación y distribución del tecnecio-99 en dos variedades de plantas de judía ***(Phaseolus vulgaris*** L.): Garrafal Nana y Contender a pH 5 y 6.8.

La captación de tecnecio-99, en forma de TcO_4^- , en Contender fue generalmente inhibida por el suministro de un exceso de dos veces la concentración control de fosfato, sulfato o molibdato, en los dos valores de pH ensayados, pH 5 y 6.8. Sin embargo, en Garrafal Nana, solamente el fosfato y el molibdato disminuyeron la absorción de tecnecio-99 a pH 5, mientras que el sulfato incrementaba su absorción. A pH 6.8 el efecto de los tres aniones considerados sobre la captación de Tc-99 era menor.

Estos resultados sugieren que diferencias en el pH y especialmente entre variedades, deberían ser consideradas cuando se utiliza la competición entre iones como un sistema para disminuir la absorción de Tc-99 o para aliviar sus efectos en las plantas.

Palabras clave: Tecnecio-99. ***Phaseolus vulgaris*** L. Nutrientes minerales. Competencia iónica.

SUMMARY

The effects of excess phosphate, sulfate and molybdate concentrations on technetium-99 uptake and distribution were studied in two varieties of bush bean plants ***(Phaseolus vulgaris*** L.) vars. Garrafal Nana and Contender, at pH 5 and 6.8.

The uptake of technetium-99, supplied as TcO_4^-, in Contender was generally decreased by a two-fold concentration excess of either sulphate, phosphate or molybdate at both pH 5 and 6.8. However, with Garrafal Nana, at pH 5, only phosphate and molybdate decreased Tc-99 uptake, while sulfate actually contributed to an increase in Tc-99 uptake. Moreover, at pH 6.8, there was less effect of the three anion treatments on technetium uptake.

These results suggest that differences in pH and especially variety, should be considered when ionic competition is used either to decrease Tc-99 uptake or ameliorate its effects in plants.

Key words: Technetium-99. ***Phaseolus vulgaris*** L. Mineral nutrients. Ion competition.

INTRODUCTION

Interest in the behaviour and effects of technetium-99 has increased recently due to the release of the element into the environment by human activities. Technetium-99 is a radioactive element produced mainly from the fission of U-238 during the energy production process in nuclear power plants. The use of Tc-99 as a tracer in nuclear medicine and the testing of atomic weapons are also some minor sources of Tc-99 contamination of the environment (Windung *et al.*, 1979; Bennássar *et al.*, 1990b).

The pertechnetate anion species (TcO_4^-) of Tc-99 is highly stable, soluble and mobile in soils and living organisms. Plants with high affinity for TcO_4^- (Landa *et al.*, 1977) as well as phytotoxic effects at low concentrations have already been reported (Cataldo *et al.*, 1978; Cataldo *et al.*, 1983; Cataldo *et al.*, 1986; Vázquez *et al.*, 1990; Bennássar *et al.*, 1991), although species and even varieties may differ in their sensitivity to Tc-99 (Mousny *et al.*, 1979; Cataldo *et al.*, 1986; Bennássar *et al.*, 1990a). Berlyn *et al.* (1980) have suggested that Tc-99 effects on plants are more the result of an interaction and competition of Tc-99 with plant mineral nutrients than radiation induced damage.

Many factors affect Tc-99 uptake by plants, such as pH, exposure time (Cataldo *et al.*, 1983; Cataldo *et al.*, 1986), plant age (Lembrechts and Desmet, 1986), and soil characteristics (Mousny and Myttenaere, 1981; Mousny and Myttenaere, 1982).

Technetium-99, like other highly mobile elements, is accumulated in the upper parts of the plants; and in these tissues it may interfere with different metabolic processes (Mousny *et al.*, 1979; Berlyn *et al.*, 1980; Cataldo *et al.*, 1983; Cataldo *et al.*, 1986). The capacity of a plant to absorb radioactive elements can be clearly visualized by the concentration ratio (CR) or coefficient of transference, which is the relationship between the activity of the element per unit tissue dry weight and the activity of the element per unit weight of the rooting medium. In plants grown on Tc-99-containing solutions in the laboratory or on Tc-contaminated soil in the field, CR values up to 1000 have been reported (Wildung *et al.*, 1977; Vandecasteele *et al.*, 1982; Sheppard *et al.*, 1983). The high CR value for Tc-99 in plants suggest the existence of a very effective uptake mechanism for this radioactive metal ion in plants. Competition kinetics studies suggest the existence for an energy mediated Tc-99 absorption process (Cataldo *et al.*, 1983). This type of transport has already been proven for other element (Fisher *et al.*, 1980).

The studies of Tc-99 interaction with plant mineral nutrients sometimes present different results and are thus difficult to compare, mostly due to the different methods used to conduct the experiments.

Berlyn *et al.*, (1980) found that Tc-99 uptake was not inhibited by manganese in *Glycine maxima*. Cataldo *et al.* (1983), also working with *Glycine maxima*, reported pertechnetate absorption to be competitively inhibited by sulfate, phosphate, selenate and molybdate, whereas no effect was found using borate, nitrate, tungstate, perrhenate, iodate or vanadate. In contrast, Myttenaere *et al.* (1986) concluded that sulfate stimulated Tc-99 uptake in *Phaseolus vulgaris* L. var. Nannus.

The purpose of the present study is to evaluate the different interactions between TcO_4^- and certain chemically similar nutrient anions, such as sulfate, phosphate and molybdate, when a full complement of nutrient solution is used and with special emphasis on pH and variety effects.

MATERIALS AND METHODS

Seeds of *Phaseolus vulgaris* L. var. Contender and var. Garrafal Nana were germinated in perlite with distilled water. After 7 to 9 days, plantlets were transferred to hydroponic culture. At this time, cotyledons were removed to avoid interference with nutrient uptake from the nutrient solution. All experiments were conducted in a glasshouse under natural light conditions.

The following treatments were set up in triplicate and at two different pH values (pH 5 and pH 6.8), in order to study the competitive relationship among the different anions and the influence of pH on anion-inhibited TcO_4^- uptake:

I. *Control treatments:* 25% Hoagland nutrient solutions at pH 5 and pH 6.8 (Hoagland and Arnon, 1950) with 0.3 g m^{-3} of TcO_4^- added as NH_4TcO_4.

II. *Competition between TcO_4^- and $H_2PO_4^-$* : Nutrient solutions as in control treatments, but supplemented with 115 g m^{-3} of $H_2PO_4^-$, added as $NH_4H_2PO_4$.

III. *Competition between TcO_4^- and SO_4^{2-}*: Nutrient solutions as in control treatments, but supplemented with 245 g m^{-3} SO_4^{2-} added as $MgSO_4.7H_2O$.

IV. *Competition between TcO_4^- and MoO_4^{2-}*: Nutrient solutions as in control treatments, but supplemented with 45 g m^{-3} MoO_4^{2-} added as Na_2MoO_4.

After 36 h exposure, plants were harvested and fresh and dry weights of the different plant organs were recorded. Technetium-99 content was analysed with a liquid scintillation spectrometer (Beckman LS 5800), after following a special digestion procedure for volatile samples (Vázquez *et al.*, 1990).

Concentration ratio (CR) values were calculated as μg ^{99}Tc g^{-1} plant dry weight / μg ^{99}Tc g^{-1} nutrient solution.

Given values are averages of three replicated experiments. Significance of difference were determined by ANOVA.

RESULTS AND DISCUSSION

Plant growth rates were not significantly affected by the different treatments during the experiment (data not shown). An experimental time of 36 h was selected so that the results of this experiment could

TABLE 1

Technetium-99 concentrations ($\mu g\ g^{-1}$ dry weight) in different organs and whole plants, percent values respect to control plants, and CR values in Phaseolus vulgaris *var. Contender.*

	pH 5		pH 6.8	
	$\mu g\ ^{99}Tc$ g^{-1} D. W.	% control	$\mu g\ ^{99}Tc$ g^{-1} D. W.	% Control
Control				
Root	39.0	100.0	27.0	100.0
Stem	10.0	100.0	9.0	100.0
Leaves	113.0	100.0	80.0	100.0
Whole plant	54.0	100.0	38.7	100.0
CR	180.0	100.0	128.9	100.0
Excess SO_4^{2-}				
Root	26.9*	68.9*	15.4*	57.1*
Stem	10.3	103.0	12.8*	142.2*
Leaves	96.9*	85.8*	60.9*	76.2*
Whole plant	44.7*	82.8*	29.7*	76.9*
CR	148.8*	82.7*	99.0*	76.8*
Excess $H_2PO_4^-$				
Root	24.3*	62.3*	18.3*	67.8*
Stem	9.5	95.0	6.0*	66.7*
Leaves	77.3*	79.8*	65.2*	81.5*
Whole plant	37.0*	68.6*	29.8*	77.1*
CR	123.2*	68.4*	99.4*	77.1*
Excess MoO_4^-				
Root	40.1	102.8	16.9*	62.6*
Stem	9.0	90.0	8.0	88.9
Leaves	95.0*	84.1*	63.4*	79.2*
Whole plant	48.0	88.9	29.4*	76.1*
CR	159.8	88.8	98.1*	76.1*

* Values marked with an asterisk are significantly different from controls (0.05% level).

be compared to previous studies. Thus we have an initial estimation of the effects of competition between TcO_4^- and other elements when plants are grown in nutrient solution containing optimum levels of mineral nutrients.

Tables 1 and 2 display the Tc-99

TABLE 2

Technetium-99 concentrations (μg g^{-1} dry weight) in different organs and in whole plants, % values respect to control plants and CR values in Phaseolus vulgaris *L. var. Garrafal Nana.*

	pH 5		pH 6.8	
	μg ^{99}Tc g^{-1} D. W.	% control	μg ^{99}Tc g^{-1} D. W.	% control
Control				
Root.	29.0	100.0	22.0	100.0
Stem.	11.0	100.0	11.0	100.0
Leaves.	81.0	100.0	75.1	100.0
Whole plant	40.3	100.0	36.0	100.0
CR	135.3	100.0	120.7	100.0
Excess SO_4^{2-}				
Root.	17.6*	60.7*	12.8*	58.3*
Stem.	16.1*	146.4*	8.5*	77.3*
Leaves.	112.0	138.3*	85.4	113.7
Whole plant	48.6*	120.4*	35.6	98.7
CR	162.3*	120.1*	118.0	97.9
Excess $H_2PO_4^-$				
Root.	6.7*	23.1*	12.3*	55.9*
Stem.	4.4*	39.5*	6.9*	62.7*
Leaves.	29.5*	36.4*	75.1	100.0
Whole plant	13.5*	33.5*	31.4	87.2
CR	45.0*	33.2*	104.7	86.7
Excess MoO_4^-				
Root.	13.2*	45.5*	14.6*	66.4*
Stem.	7.2*	65.5*	7.5*	68.2*
Leaves.	37.0*	45.7*	70.4	93.7
Whole plant	19.1*	47.4*	30.8*	85.5*
CR	63.7*	47.1*	102.8*	85.2*

* Values marked with an asterisk are significantly different from controls (0.05 % level).

content of the different plant organs and on a whole plant basis in Contender (Table 1) and Garrafal Nana (Table 2) in $\mu g\ g^{-1}$ dry weight, percentage with respect to controls, and the CR values in the different treatments considered. Within both varieties, Contender and Garrafal Nana and at both pH levels, Tc-99 mainly accumulated in leaves and to less extent in roots and stems. In control plants, both Tc-99 concentrations and CR values generally were higher for the pH 5 than for the pH 6.8 treatment.

Contender had the most uniform behaviour in regards to uptake inhibition by anions (Table 1). In this variety, at pH 5, the transfer of pertechnetate from the solution to plant tissues, expressed as CR values, was inhibited by sulfate, phosphate and molibdate to 82.7, 68.4 and 88.8% of control, respectively. Anion effects were similar in solutions at pH 6.8. Excepting the molibdate treatment at pH 5, Tc-99 concentrations in roots were the most affected.

In Garrafal Nana (Table 2), the competition between pertechnetate and sulfate, phosphate and molibdate demonstrated different tendencies, depending on the anion and pH values considered. At pH 5, an excess of sulfate decreased the concentration of Tc-99 in roots, but increased Tc-99 concentrations in stems and leaves. At pH 6.8, excess sulfate decreased Tc-99 concentrations in roots and stems but did not significantly affect the transfer of Tc-99 from the solution at the whole plant level. Our results on the influence of sulfate on Tc levels and CR values in both varieties suggest, that sulfate in addition to an inhibitory effect on Tc-99 uptake in plants, may enhance the translocation of this radioactive metal to upper plant parts. These opposite actions of sulfate on Tc uptake and translocation which may explain some of the contradictory results reported in the literature, deserve further investigations, moreover taking into account indirect experimental evidences from others which indicate that Tc-99 may act in plants as a nonfunctional sulfate analogue (Cataldo *et al.*, 1986).

In Garrafal Nana at pH 5, excess phosphate and molibdate severely inhibited Tc-99 uptake as shown by the CR values, which were only 33.2% and 47.1% of the control, respectively (Table 2). Nevertheless, in this cultivar the inhibitory effect of these anions significantly depended on the solution pH and was much lower at pH 6.8. At pH 6.8 the excess of anions tested did not affect the Tc-99 concentrations in leaves, but only in roots and stems. The above results are consistent with the studies conducted by others (Cataldo *et al.*, 1983) on the pertechnetate absorption kinetics. They found Tc-99 uptake controlled by a very efficient carrier-mediated transport mechanism present for these anions.

Further studies on sulfate, phosphate and molibdate levels have to be undertaken in order to show, if the different behaviour of the varieties obey to varietal differences in the capacity to absorb and translocate these anions at different pH values, or to genetical differences in the response to Tc-99.

CONCLUSIONS

Our study shows that anions such as sulfate, phosphate and molibdate inhibit the uptake of Tc-99 in bean plants. Moreover, these anions, especially sulfate, influence the translocation of Tc-99 in plants. The effect of anions on Tc-99 uptake and translocation in plants is somehow modulated by different factors, such as pH and plant variety. These factors should be considered in case mineral nutrient interaction is used to either inhibit Tc-99 uptake by plants or ameliorate Tc-99 effects on plants.

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