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Research Report

Arsenic Uptake and Partitioning in Grafted Tomato Plants

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Abstract. Arsenic is a toxic and cancerogenic metalloid that poses a threat to food crop consumption. Previous studies have shown that grafting vegetables onto certain rootstocks may restrict the uptake of some toxic metals, such as cadmium, lead, and so on, but no such study has investigated the uptake of arsenic. The aim of this work was to determine the following: i) if grafting can influence and reduce arsenic translocation in the root and/or aerial organs; ii) how tomato plants irrigated with arsenic-enriched nutrient solution (100 $\mu g \cdot L^{-1}$) accumulate this metalloid; and iii) if arsenic poses a potential risk to fruit quality. We found that differences in plant growth and the qualitative traits of fruits were mainly related to the adopted rootstock rather than to the addition of arsenic. Grafting influenced metalloid accumulation in roots and its translocation from roots to shoots and fruits. Tomato plants accumulated arsenic in their roots, and only a small portion was translocated to shoots and fruits, making the risk for human consumption negligible. Therefore, the uptake of this toxic element and its translocation are influenced by the rootstock utilized.

Additional key words: bioaccumulation, grafting, heavy metal, *Solanum lycopersicum*, translocation

Introduction

Arsenic (As) is a metalloid that is widely present in the environment for both geogenic and anthropogenic reasons, such as mining, industrial, and agricultural activities (Meharg et al., 1994). In the past years, this metalloid has received worldwide attention because of its toxicity for both humans (arsenic and its compounds are classified are carcinogenic and listed in group 1 by the International Agency for Research into Cancer) and plants. In plants, roots are usually the first tissue exposed to As, which inhibits their extension and growth. At sufficiently high concentrations, As interferes with critical metabolic processes, such as oxidative phosphorylation and ATP biosynthesis, which can lead to plant death (Finnegan and Chen, 2012). Moreover, As translocation to the shoot can severely inhibit plant growth and productivity (Garg et al., 2011).

Arsenic occurs in the environment in two main oxidation states, trivalent and pentavalent, existing both in organic and inorganic forms. The most soluble compounds are the inorganic forms: arsenate, As(V) and arsenite, As(III). The concentration, chemical forms, and oxidation state of As (Carbonell-Barrachina et al., 1998) and the soil microbial community (Stazi et al., 2015) play an important role in the behaviour of the many As compounds in the environment and their uptake in plants, affecting their accumulation in products for human consumption (Jia et al., 2014). Moreover, uptake and transport systems vary from species to species (Walsh et al., 1975; Marmiroli et al., 2014). In higher plants, arsenate is taken up and translocated via the high-affinity Pi transporter system due to its chemical analogy with Pi (Zhao et al., 2009). After uptake as arsenate, one of the tolerance mechanisms utilized by plants, it is transformation into arsenite via a reduction process, which is mediated by glutathione and glutathione S-transferase (Sharma, 2012).

One method for reducing As accumulation in food crops is the selection of genotypes with low As uptake rates. It has been widely demonstrated that grafting onto specific genotypes can reduce the toxic effect of metals on vegetables (Rouphael et al., 2008; Savvas et al., 2010), because the root structure and uptake efficiency are determined by rootstocks. Plants grafted onto certain rootstocks may exhibit dissimilar abilities to take up nutrients and other non-nutrient elements (Savvas et al., 2010). These mechanisms may also be influenced by the shoot through alterations in the transport of some metabolites, which are initiated by hormonal messengers (Zhou et al., 2007; Albacete et al., 2009).

According to European standards, the limit of As in drinking water is 10 $\mu g \cdot L^{-1}$ (Dir. 98/83/EC), but many municipalities have banned the use of water with this quantity of As for crop irrigation, and there is a lack of legislation regulating As levels in irrigation water. In addition, there are no clear rules by the World Health Organization (WHO) on acceptable levels for in agro-food products. Therefore, WHO is keeping a close watch on crops that can accumulate As in the environment.

Tomato is one of the main horticultural crops in both Europe and the US, but its fruits can be compromised by As contamination. Several researchers have studied the assimilation of As in polluted soil and irrigation waters, highlighting the capability of the crop to store As in roots and translocate only a small portion to the shoot, according to genotype (Burlò et al., 1999; Marmiroli et al., 2014). However, the mechanisms underlying the impediment of heavy metal uptake and translocation to the shoot by some rootstocks are still unclear (Savvas et al., 2010). Thus, investigating the use of interspecific tomato hybrids as rootstocks could be useful for understanding the behaviour of different grafting combinations.

The main objective of this study was to investigate the effects of rootstock on the uptake and partitioning of available As. To this end, we tested three grafting combinations exposed to 100 $\mu g \cdot L^{-1}$ As in the nutrient solution. This level is commonly found in areas naturally polluted by As, such as volcanic areas and thermal springs; nevertheless, this concentration is much higher than the legal limit for drinkable water (10 $\mu g \cdot L^{-1}$). Specifically, we investigated the following: i) how uptake and translocation of As in tomato plants vary with different concentrations of the element in the rhizosphere; ii) if and how rootstocks influence As content in tomato organs; and iii) if fruits accumulate high concentrations of As when it is added to nutrient solution.

Material and Methods

Plant material, Treatments, and Growth Conditions

The trial was conducted in Spring 2014 in a shaded shelter in a farm located in the coastal area of Catania province, Italy (37.31°N; 15.40°E; 20 m asl). 'Caramba' F_1 tomato plants were grafted onto two tomato interspecific hybrids (*Solanum lycopersicum* × *Solanum habrochaites*) and self-grafted, thus yielding three grafting combinations: C/C, 'Caramba' F_1/'Caramba' F_1 (self-grafted plants); C/M, 'Caramba' F_1/'Maxifort' F_1; and C/H, 'Caramba' F_1/'He-Man' F_1. The three grafting-combinations were irrigated with a nutrient solution containing 100 $\mu g \cdot L^{-1}$ of As (by adding NaH_2AsO_4, Sigma Aldrich). Self-grafted plants irrigated with As-free nutrient solution were adopted as a control.

Grafted seedlings were transplanted on 10th May at the third true-leaf stage into 9.5 L volume pots filled with sand at a planting density of six plants/m^2. Nutrient solution was prepared according to Sonneveld and Straver (1992). The pH of each nutrient solution was adjusted to 5.9, and the nutrient solutions were pumped from independent tanks at a flow rate of 4 $L \cdot h^{-1}$ using a drip irrigation system, with one emitter per plant. A leaching fraction of >70% was adopted in order to reduce the accumulation of salts in the substrate.

During the trial, the climate conditions were constantly monitored and recorded on a data logger (CR10X; Campbell Scientific Ltd., Loughborough, UK). The mean air temperature was 26°C, the relative humidity (RH) was approximately 62%, and the mean global radiation was 20.15 $MJ \cdot m^{-2}$.

Plant Growth and Fruit Qualitative Traits

To evaluate the effects of As and grafting treatments on plant growth and fruit qualitative traits, the plants were sampled at the end of July (77 days after transplanting, DAT). Plant tissues were oven dried at 70°C to obtain dry weight (DW). From each replicate, eight representative marketable fruits were analyzed for the following parameters: unit weight (g), firmness, chromatic coordinates, titratable acidity, and soluble solids. Firmness was measured with an electronic dynamometer (TA-XT2, Stable Micro Systems Ltd., Godalming, UK) and is expressed as the force (g) needed to produce a deformation of 2 mm with respect to the longitudinal axis of the fruit. Chromatic coordinates (lightness L*, chroma C*, hue angle h°) were determined on tomato skin using a Minolta chroma meter (CR-200, Konica Minolta Business Solutions, Tokyo, Japan). The titratable acidity was obtained by titration with a solution of 0.1 N NaOH ($g \cdot L^{-1}$ of citric acid). Soluble solids (°Brix) were measured with a digital refractometer with automatic compensation for temperature (Brix PR-1, Atago Co., Ltd., Tokyo, Japan).

Soil Characterization

Sandy soil was used as a substrate and characterized at the beginning of the trial before As treatment. Soil pH was measured in water (pH_{H2O}) and in 1N KCl (pH_{KCl}) with a soil: solution ratio of 1:2.5 (w/v); the moisture content was determined by oven drying the soil samples at 105°C. The total organic C (TOC) and total N (TN) contents were measured by dry combustion (EA-1110 CE instrument, Milan, Italy). Cation exchange capacity (CEC) was determined according to Gillman (1979). Results are expressed as $cmol_{(+)}$ kg^{-1} of soil. Available P concentration was determined according to Olsen and Sommers (1982). The amount of bioavailable As, i.e., the labile fraction that can be taken up by the plants and soil organisms, was evaluated after soil extraction with 0.05 M $NH_4H_2PO_4$ (20°C/16 h) (Wenzel et al., 2001). The As concentration in digested soil and in the liquid fractions was measured with an ICP-OES (8000 DV, PerkinElmer, Shelton, CT, USA).

Arsenic and Phosphorous Chemical Analyses

Roots, shoots, and fruit were analyzed to determine the quantity of As and P. The reagents used were super pure for trace analysis. The accuracy of the measurements was assessed using standard reference materials trace metals: Loamy Sand 3 (CRM034-Fluka) and tomato leaves (SRM 1573a).

Acid digestion of the samples was performed using a microwave oven (Mars plus CEM, CEM Corporation, Matthews, NC, USA) operating at an energy output of 1600W. All samples were divided into small pieces, washed with water, and dried at 65°C for 72 h. Approximately 500 mg of sample was inserted directly into a 100 mL PFA HP-500 Plus digestion vessel, and 2 mL of 30% (m/m) H2O2, 0.5 mL of 37% HCl, and 7.5 mL of 69% HNO3 solution were added to the vessel. The heating program was performed in a single step. The temperature linearly increased from 25 to 180°C in 37 min and was held at 180°C for 15 min. After the digestion procedure and subsequent cooling, the digested samples were diluted to a final volume of 20 mL with Milli-Q water. Blanks were prepared in each lot of samples. All experiments were performed in duplicate. An ICP-OES with an axially viewed configuration equipped with an ultrasonic nebulizer was used for the As and P determinations. For detection, the frequency with the lowest interference was chosen, with a high analytical signal-to-background ratio: the As line was at 193.69 nm and P was at 213.62 nm.

Bioaccumulation and Translocation Factors

The water-to-root bioaccumulation factor (BAF) of a metalloid describes the ability of a plant to accumulate the element from water and soil. The BAF was calculated as the ratio of As concentration "on a root dry weight basis" to the corresponding total concentration of As in the nutrient solution.

The root-to-plant translocation factor (TF) describes the movement and distribution of As from the root to the aerial parts of the plant. The TF is operationally calculated as the ratio between the As concentration in a plant (in shoots or fruits) on a DW basis and the corresponding total concentration in roots at the end of the experiment (Liu et al., 2014).

Statistical Design

A randomized block design with five replicates per treatment was used (six plants per replicate). Three plants per replicate were sampled at final harvest for dry weight measurements. Qualitative traits were determined on selected fruits (eight per replicate). Tissue analysis was conducted in triplicate. Data were statistically analyzed using STATISTICA v.10 (StatSoft Polska). For each one-dimensional variable, ANOVA was utilized, and for all variables, the Tukey HSD test was used. Homogeneous univariate pair comparison was performed at a significance level of $p \leq 0.05$.

Results and Discussion

Plant Growth and Fruit Qualitative Traits

The dry weights of different organs did not differ between the two C/C combinations in relation to As content in the nutrient solution (Table 1). This result is in contrast to the findings of Burló et al. (1999), who found that tomato plants treated with arsenite exhibited a higher DW than the controls, although at a higher As concentration than was utilized in

Table 1. Shoot (leaf, stem), fruit and root dry weight (g), and qualitative characteristics of 'Caramba' fruits of grafted tomato plants subjected to different treatments at harvesting of the first truss (77 DAT, days after transplanting)

Treatment^z	Plant DW (g per plant)			Unit weight (g)	Dry matter (%)	Firmness (g)^y	Titratable acidity ($g{\cdot}L^{-1}$ citric acid)	Soluble solids (°Brix)
	Shoot	Fruit	Root					
C/C	57.9 b	75.1	8.12	162	5.54 b	1313 a	5.76 a	2.80 ab
C/C + As	62.9 b	85.5	8.81	169	5.86 ab	1312 ab	4.88 ab	2.51 b
C/M +As	65.2 ab	84.9	7.61	168	5.69 ab	1089 b	4.66 b	3.10 a
C/H +As	73.6 a	84.9	9.34	164	5.98 a	1502 a	5.76 a	2.72 ab
ANOVA^x	*	ns	ns	ns	*	*	**	*

^{z}+As: plants irrigated with As-enriched nutrient solution (100 $\mu g{\cdot}L^{-1}$); C/C: 'Caramba'/'Caramba'; C/M: 'Caramba'/'Maxifort'; C/H: 'Caramba'/'He-Man'.

^{y}1 gram-force is equal to 0.00980665 Newton (N).

^{x}*, **, and *** represent significance at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively; ns, not significant. For each column, mean values labelled with the same letter do not differ significantly.

the current study. For grafting combinations, the use of ‘He-Man’ as rootstock (known for its high vigor) led to an increase in shoot DW of approximately 17% compared to plants grafted onto ‘Caramba’ (C/C+As). No difference was detected for fruit and root DW. Nevertheless, the highest shoot DW found in C/H plants could be attributed to the more vigorous root system, which increases nutrient and water uptake, enhancing plant growth (Öztekin et al., 2009). The unit weights of tomato fruits did not differ among treatments (Table 1). In addition, the dry matter % did not differ between the C/C grafted plants (control and +As) and the grafting combinations under As treatment. Firmness values did not differ between C/C combinations irrespective of As treatment, whereas among rootstocks, grafting onto ‘He-Man’ resulted in increased firmness compared with plants grafted onto ‘Maxifort’ (+38%). The effect of grafting on firmness was previously tested in other vegetable crops such as watermelon (Yetisir et al., 2003) and melon (Colla et al., 2006). The effect is probably due to the variation in cell turgor, as well as the chemical and mechanical properties of the fruit cell wall resulting from the increased biosynthesis of endogenous hormones (Rouphael et al., 2010), which occurred due to the high vigor of ‘He-Man’ rootstock. The treatment with and without As-enriched nutrient solution has shown no difference on tritatable acidity for C/C combinations. Among rootstocks, the highest value was found in the C/H combination (+24% compared to C/M grafted plants; Table 1). Soluble solids did not differ between C/C combinations. For rootstocks, the highest values were recorded in C/M plants compared to C/C+As, which contradicts the previous finding that grafting of tomato does not affect total soluble solids (Di Gioia et al., 2010). It is likely that at harvest time, fruits of the C/M grafting combination were riper than those of the other combinations, also resulting in the lowest titratable acidity.

Arsenic Phytoavailability and Soil Chemical Properties

Soil physical and chemical properties are important characteristics affecting As bioavailability (Table 2). The soil used in our investigation was sandy and characterized by low concentrations of clay and organic matter, the soil components that most strongly bind to metalloids. The concentration of total As in potted-soil was 10.97 µg·g^{-1}. Since Violante et al. (2006) reported a release of 52% of the As adsorbed onto the surfaces of gibbsite after the addition of phosphate solution, we expected a high level of metalloid release from the sandy soil in our study after the addition of nutrient solution. Extraction of the soil with 0.05 M $NH_4H_2PO_4$ led to the release of 58% of As into the soil solution (the labile fraction), corresponding to 6.43 µg·g^{-1} of the element bioavailable for plant uptake.

Table 2. Physical and chemical properties of soil irrigated with As nutrient solution

Soil properties	
Texture	Sandy soil
pH (H_2O)	7.5
pH (KCl)	7.3
Organic Carbon (%)	0.05%
CEC (cmol(+)kg^{-1})	9.06
Available P (P_2O_5, mg·kg^{-1})	1.5
Total Nitrogen (%)	< LD^{z}
Total As (µg·g^{-1})	10.97
Bioavailable As (µg·g^{-1})	6.43

^{z}LD: limit of detection

As Uptake and Partitioning: Effect of As Concentration in the Rhizosphere

We compared As levels in roots, shoots, and fruits of self-grafted combinations that were irrigated with As-enriched nutrient solution (C/C+As) versus the control (C/C) (Table 3). The concentration of As was enhanced by treatment with enriched nutrient solution, with an increase of 3.71-fold in C/C+As compared with the control for roots and 1.60-fold for both shoots and fruits (Table 3). Most of the metalloid that had accumulated in C/C+As was confined to the roots

Table 3. Amount of As (µg·g^{-1} DW) and P (µg·g^{-1} DW) in different organs of grafted tomato plants under different treatments

Treatment^{z}	As			P		
	Root	Shoot	Fruit	Root	Shoot	Fruit
C/C	6.52 ± 0.14 c	1.04 ± 0.04 c	0.05 ± 0.004 c	3.40 ± 0.14 a	4.16 ± 0.16 ab	6.30 ± 0.08 a
C/C + As	24.21 ± 0.80 a	1.67 ± 0.04 a	0.08 ± 0.010 b	2.29 ± 0.05 d	3.69 ± 0.06 c	6.12 ± 0.04 a
C/M +As	8.11 ± 0.35 c	1.20 ± 0.05 b	0.11 ± 0.007 a	2.98 ± 0.03 b	4.37 ± 0.12 a	5.38 ± 0.14 b
C/H +As	11.72 ± 0.08 b	1.54 ± 0.03 a	0.09 ± 0.006 b	2.62 ± 0.06 c	3.85 ± 0.09 bc	6.57 ± 0.19 a
ANOVA^{y}	***	***	**	***	***	***

^{z}+As: plants irrigated with As-enriched nutrient solution (100 µg·L^{-1}); C/C: ‘Caramba’/‘Caramba’; C/M: ‘Caramba’/‘Maxifort’; C/H: ‘Caramba’/‘He-Man’.

^{y}*, **, and *** represent significance at $p \le 0.05$, $p \le 0.01$, or $p \le 0.001$, respectively; ns, not significant. For each column, mean values labelled with the same letter do not differ significantly.

Table 4. Translocation factor for arsenic in grafted tomato plants under different treatments

Treatment[z]	$[As]_{shoot}/[As]_{root}$	$[As]_{fruit}/[As]_{root}$
C/C	0.16 ± 0.01 a	0.010 ± 0.001 b
C/C + As	0.07 ± 0.01 c	0.003 ± 0.0004 c
C/M +As	0.16 ± 0.01 a	0.014 ± 0.001 a
C/H +As	0.13 ± 0.003 b	0.007 ± 0.001 b
ANOVA[y]	***	***

[z] +As: plants irrigated with As-enriched nutrient solution (100 $\mu g \cdot L^{-1}$); C/C: 'Caramba'/'Caramba'; C/M: 'Caramba'/'Maxifort'; C/H: 'Caramba'/'He-Man'.

[y] *, **, and *** represent significance at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively; ns, not significant. For each column, mean values labelled with the same letter do not differ significantly.

(24.21 $\mu g \cdot g^{-1}$), and only a small portion was translocated to aerial parts (1.67 $\mu g \cdot g^{-1}$ and 0.08 $\mu g \cdot g^{-1}$ for shoots and fruits, respectively). Similar result were obtained for the control plants (C/C), where the highest levels of As were stored in roots (Table 3). Analysis of translocation factor (TF) values, a representative index used to indicate the shift of metalloid from roots to shoots or fruits, confirmed that the As concentration in roots increased more than that in shoots (Table 4). We observed significant differences between TF in C/C combinations (control and +As), indicating that the lower the amount of As in the roots, the higher the amount of this metalloid transferred to shoots and fruits. This result may be due to the phosphate channels' affinity for arsenic compounds: if these compounds are not present as arsenate, they are stored in roots and not translocated to the shoot. This finding likely indicates that As accumulates in the root system in its reduced form, namely arsenite (Zhao et al., 2010); this phenomenon may constitute the physiological mechanism by which tomato plants limit the shift of As into their aerial parts. To evaluate the affinity between As and phosphate, we calculated the P concentration ($\mu g \cdot g^{-1}$) in roots, shoots, and fruits, as shown in Table 3. In the presence of As in the nutrient solution, we observed a significant decrease in P concentration, with a decrease of 32% in roots and 11% in shoots in C/C+As versus C/C plants.

Effect of Grafting Combinations

To better investigate the effect of rootstock on As uptake in roots and subsequent transport to edible parts of the plant, we compared the levels of As uptake and partitioning in various grafting combinations, which were expressed in terms of concentration, as shown in Table 3. We compared the As uptake abilities of the tested rootstocks. Grafting combination C/M was more efficient than C/H and C/C at limiting As in its roots, with a concentration 31% and 66% lower than that of 'He-Man' and 'Caramba' rootstocks, respectively. The differential uptake of the grafted plants could be attributed to the specific root exudates produced by the rootstocks (Bergqvist et al., 2014) or to differences in their roots, which could have differential abilities for limiting arsenate uptake (Xu et al., 2007). In addition, the shoot As accumulation significantly differed among grafting combinations, showing an analogous pattern to that of roots. The lowest value was found in the C/M combinations, whereas this value did not differ between C/H and C/C grafted plants. In contrast to the results for roots and shoots, the As level in fruits of the C/M combinations were 37% higher than those of C/C and C/H fruits.

The fruits were not heavily contaminated by As. The lowest concentrations were found in the C/H and C/C+As combinations, which did not significantly differ. On the contrary, the highest level was detected in fruits of C/M grafted plants, reaching a concentration of 0.11 $\mu g \cdot g^{-1}$ As measured on a DW basis (i.e., approximately 0.0063 $\mu g \cdot g^{-1}$ FW). The trends observed for FW and DW were similar. The consumption of 1 kg of fresh tomato containing this amount of As would involve the intake of approximately 6.3 μg of As (0.9 $\mu g \cdot kg^{-1}$ body weight of an average adult). However, current international guidelines (European Food Safety Authority, 2010) indicate that the intake of 0.3 to 8 $\mu g \cdot kg^{-1}$ b.w. per day is tolerable.

Bioaccumulation factor (BAF) reflects the accumulation of As in plants from As available in the nutrient solution. The higher the BAF, the greater the ability of the root to store As. In the current study, plants grafted onto 'Caramba' showed the highest BAF, followed by plants grafted onto 'He-Man' and 'Maxifort' (Fig. 1).

For all grafting combinations, the TF (Table 4) to shoots was much higher than that to fruits. In addition, for the self-grafting combination, we found that the As concentration in the roots also had an effect: the lower the level of As in roots, the higher the metalloid levels in shoots and fruits. Among combinations, C/M had the lowest As levels in roots but the highest levels in fruits (Table 3). These As levels were shifted, with a higher TF from roots to fruits in the C/M combination than in the two other combinations (Table 4).

The capacity for P accumulation differed among rootstocks

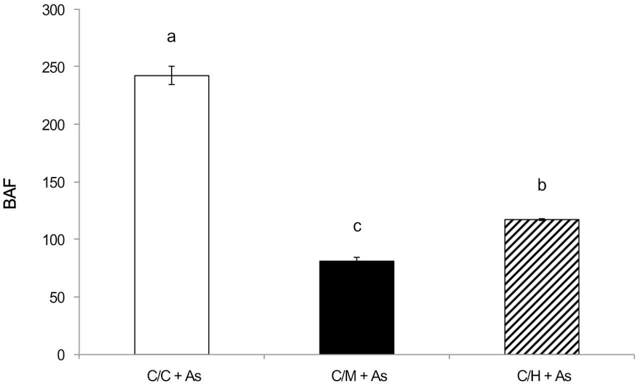

Fig. 1. Bioaccumulation Factor (BAF) of grafting combinations (C/C: 'Caramba'/'Caramba'; C/M: 'Caramba'/'Maxifort'; C/H: 'Caramba'/ 'He-Man') exposed to enriched As nutrient solution (+As, 100 $\mu g{\cdot}L^{-1}$). For each column, mean values (n = 6) labelled with the same letter do not differ significantly.

(Table 3), showing an opposite trend to that of As uptake. Roots of the C/M combination exhibited the highest P concentration (whereas it had the lowest value for As), followed by C/H (-12%) and C/C (-23%); these differences were significant. The same trend was exhibited in shoots and fruits, with the highest P concentration for C/M (which had the lowest value for As), followed by C/H (-12%) and C/C (-16%). Fruits of the C/M combination had the lowest P concentrations, even though they had higher P concentrations than roots. In this case, C/M had the lowest As concentration in roots and the highest translocation of As in fruits. The grafted plants had significantly lower amounts of metalloid in their aerial parts compared to roots.

In this study, we investigated the uptake, accumulation, and translocation of arsenic in grafted tomato plants irrigated with an As concentration widely found in irrigation water in naturally polluted areas (100 $\mu g{\cdot}L^{-1}$). The results show that As compounds mainly accumulate in the root, whereas only a small portion is translocated to shoots and fruits. Rootstocks differentially affected the uptake and distribution of As in plant tissues. The use of 'Maxifort' rootstock increased As accumulation in tomato fruits. However, our findings indicate that fruits did not accumulate hazardous levels of As. Arsenic accumulation and translocation exhibited the opposite trend to that of phosphorus, confirming the antagonism between the two elements in all rootstocks. Considering the differential effects of different rootstocks on As uptake and partitioning, further investigations should focus on the As response of the most highly utilized rootstocks for crop cultivation in contaminated soils or with polluted irrigation waters. Studies should also focused on understanding the influence of grafting on As speciation and, consequently, on its translocation and accumulation in plant tissues.

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