An effective rhizoinoculation restraints arsenic translocation in peanut and maize plants exposed to a realistic groundwater metalloid dose

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ABSTRACT: Groundwater with high arsenic (As) concentration constitutes a serious problem for crops, since roots can accumulate the metalloid acting as the first stage of As distribution in the trophic chain. The aim of this research was to elucidate the impact of a realistic As(V) dose in peanut and maize plants and to determine the contribution of plant growth promoting bacteria (PGPB) to metalloid translocation in both crops. The results obtained revealed that rhizoinoculation of plants exposed to metalloid, contributed not only to improve growth but also to reduce As transport to shoots. Hence, inoculation of peanut and maize with the correct PGPB partner prevents metalloid translocation in plants avoiding possible fruit contamination.

1 INTRODUCTION

Arsenic (As) is a harmful metalloid that impacts on crops acting as the first stage of As distribution in the trophic chain. In Córdoba province (Argentina), 90% of the region is affected by groundwater As concentrations that exceeds the maximum allowed level in drinking water (FAO, 2015), being arsenate (As(V)) the prevalent arsenic form (Blarasin *et al.*, 2014). Peanut and maize plants constitute important crops in Córdoba, as they represent approximately 90% of the Argentinean production. These crops establish interactions with plant growth promoting bacteria (PGPB) improving yield (Glick, 1995). Given the As amounts determined in groundwater, the metalloid absorption by crop plants could represent an agricultural and human health problem. Therefore, our experiments were conducted to elucidate the impact of a realistic As(V) dose in peanut and maize plants growth and to determine the contribution of PGPB to metalloid translocation in both plants.

2 METHOD/EXPERIMENTAL

2.1 Bacterial strains

Bradyrhizobium sp. SEMIA6144 and *Bradyrhizobium* sp. C-145 strains were obtained from MIRCEN (Brazil) and INTA (Argentina), respectively. *Azospirillum brasilense* AZ39 and *A. brasilense* CD (ATCC 29710) were provided by IMIZA-INTA (Argentina) and EMBRAPA (Brasil), respectively. *Bradyrhizobium* strains were cultivated in liquid YEM medium

(Vincent, 1970) and *Azospirillum* strains in liquid Nfb medium (Dobereiner, 1988).

2.2 Plant material and experimental design

Peanut and maize seeds were supplied by “El Carmen S.A.” and DEKALB respectively. Seeds were surface sterilized (Vincent, 1970) and pre-germinated seeds were transferred to a Leonard Jar system containing sterile substrate sand:perlite (2:1) with Hoagland’s nutrient solution (Hoagland and Arnon, 1950) devoid of As(V) (control) or containing 3 µM As(V), supplied as Na₂HAsO₄·7H₂O (the metalloid concentration found in groundwater of some areas of Córdoba). Plants were divided in two groups (non-inoculated and inoculated) and grown in a controlled environment for 30 days. At harvest, growth and nodulation variables and nitrogen content (by Kjeldahl method) were determined.

2.3 Arsenic accumulation and translocation in plant tissues

Metalloid concentration was determined in peanut and maize shoots, roots and nodules (legume) by using an inductively coupled plasma mass spectrometry (ICP-MS) (Sobrinho-Plata *et al.*, 2009). The translocation factor (TF) was calculated as Singh and Agrawal (2007).

2.4 Statistical analysis

Experiments were conducted in a completely randomized design and repeated three times. The data were

analyzed using ANOVA and Duncan's test at $P > 0.05$. Prior to the test of significance, normality and homogeneity of variance were verified using the modified Shapiro-Wilk and Levene tests, respectively.

3 RESULTS AND DISCUSSION

3.1 Arsenic impact on peanut and maize growth

Peanut plants exposed to As(V) showed a significant reduction in shoot dry weight only in non-inoculated plants, without differences in the root dry weight (Table 1). Nodule number and dry weight of peanut plants were reduced by As. Nevertheless, the comparison between bradyrhizobial strains revealed that *Bradyrhizobium* sp. C-145 had a better behavior than *Bradyrhizobium* sp. SEMIA6144 since these variables were significantly higher in both control and treated condition. In the control treatment, nitrogen content was similar in plants inoculated with either bradyrhizobial strain, and significantly greater than in non-inoculated plants. However, As(V) addition caused a reduction of nitrogen content only in plants inoculated with *Bradyrhizobium* sp. SEMIA6144. In maize plants, all growth variables as well as nitrogen content were decreased by metalloid addition irrespective of the inoculation condition tested. Remarkably, *Azospirillum* strains allowed greater bi-ological nitrogen fixation (BNF) in plants, as determined by nitrogen content, compared to non-inoculated plants even when the crops were exposed to the metalloid (Table 1). In support of our results a significant reduction of soybean, peanut, lupin and maize growth were observed when exposed to As(V), being the generation of reactive oxygen species the main reason of cellular toxicity (Bianucci *et al.*, 2017, 2018; Finnegan & Chen, 2012; Lu *et al.*, 2017; Vázquez-Reina *et al.*, 2005). In addition, reduction of nodulation variables was also observed in lupin and soybean plants exposed to As (Bianucci *et al.*, 2018; Carpena *et al.*, 2006). It is known that the interaction established between peanut or maize with PGPB, as used in this research, is important since it fulfils a plant's N demand via the BNF process (Glick, 1995). However, reports evaluating the impact of As in the symbiotic interaction between peanut and rhizobia is scarce, even more in the maize-PGPB interaction. According to our results, it is possible to suggest that the inoculation of peanut with *bradyrhizobia* or maize with *Azospirillum* contribute to enhance growth variables in crops exposed to a realistic As groundwater dose. In this sense, *Bradyrhizobium* sp. C-145 and *A. brasilense* Az39 strains had the better behavior among tested strains.

3.2 Arsenic accumulation and translocation in peanut and maize plants

Arsenic accumulation by peanut plants was mainly detected in roots, followed by nodules and finally in shoots, regardless of the inoculation condition (Table 2). Metalloid content in shoots of plants

Table 1. Impact of arsenate on peanut and maize growth.

		Shoot dry weight (g)		Root dry weight (g)		Nodule number plant ⁻¹		Nodule dry weight (g plant ⁻¹)		Nitrogen content (mg g ⁻¹ dry weight)	
		Control	As(V)	Control	As(V)	Control	As(V)	Control	As(V)	Control	As(V)
Peanut	non-inoculated	0.93±0.05 ^{A1}	0.75±0.02 ^{A2}	0.21±0.004 ^{A1}	0.20±0.041 ^{B1}	nd	nd	nd	nd	17.09±2.75 ^{A1}	16.19±1.26 ^{A1}
	<i>Bradyrhizobium</i> sp. SEMIA 6144	0.92±0.14 ^{A1}	0.75±0.03 ^{A1}	0.23±0.018 ^{A1}	0.24±0.009 ^{A1}	27.70±1.10 ^{A1}	21.20±1.00 ^{A2}	0.04±0.004 ^{A1}	0.03±0.001 ^{A2}	26.24±2.49 ^{B1}	17.01±0.40 ^{A2}
	<i>Bradyrhizobium</i> sp. C-145	1.05±0.14 ^{A1}	0.85±0.04 ^{A1}	0.21±0.012 ^{A1}	0.23±0.006 ^{A1}	54.00±4.70 ^{B1}	25.20±2.00 ^{B2}	0.06±0.003 ^{B1}	0.03±0.001 ^{A2}	26.03±0.48 ^{B1}	25.22±1.13 ^{B1}
Maize	non-inoculated	0.22±0.01 ^{B1}	0.17±0.01 ^{B2}	0.43±0.02 ^{B1}	0.28±0.02 ^{A2}	nd	nd	nd	nd	17.93±0.55 ^{C1}	13.61±0.85 ^{B2}
	<i>Azospirillum</i> <i>brasilense</i> Az39	0.31±0.01 ^{A1}	0.24±0.02 ^{A2}	0.46±0.05 ^{AB1}	0.27±0.03 ^{A2}	nd	nd	nd	nd	25.27±1.09 ^{A1}	16.48±0.21 ^{A2}
	<i>Azospirillum</i> <i>brasilense</i> CD	0.28±0.02 ^{A1}	0.22±0.01 ^{A2}	0.55±0.03 ^{A1}	0.24±0.01 ^{A2}	nd	nd	nd	nd	21.28±0.65 ^{B1}	15.63±0.44 ^{A2}

Data represent the mean ± SE ($n = 10$). Different letters in each column indicate significant differences among inoculation conditions for each treatment and different numbers on each row indicate significant differences among treatment for each inoculation condition according to the Duncan's test ($P < 0.05$). Nd: not determined.

Table 2. Arsenic accumulation and translocation in peanut and maize plants exposed to As(V).

		Arsenic accumulation ($\mu\text{g g}^{-1}$ dry weight)			
		Shoot	Root	Nodule	TF
Peanut	non-inoculated	18.64 $\pm$ 0.62 ^b	64.45 $\pm$ 1.22 ^c	nd	0.29 $\pm$ 0.007 ^a
	<i>Bradyrhizobium</i> sp. SEMIA 6144	15.04 $\pm$ 0.39 ^b	178.85 $\pm$ 4.00 ^a	130.70 $\pm$ 10.85 ^a	0.08 $\pm$ 0.002 ^b
	<i>Bradyrhizobium</i> sp. C-145	34.41 $\pm$ 2.25 ^a	121.47 $\pm$ 4.34 ^b	81.70 $\pm$ 1.24 ^b	0.28 $\pm$ 0.017 ^a
Maize	non-inoculated	9.14 $\pm$ 0.39 ^a	7.21 $\pm$ 0.16 ^c	nd	1.26 $\pm$ 0.046 ^a
	<i>Azospirillum brasilense</i> Az39	7.52 $\pm$ 0.34 ^b	14.19 $\pm$ 0.55 ^b	nd	0.53 $\pm$ 0.015 ^b
	<i>Azospirillum brasilense</i> CD	10.16 $\pm$ 0.23 ^a	33.27 $\pm$ 0.83 ^a	nd	0.30 $\pm$ 0.003 ^c

Data represent the mean $\pm$ SE (n = 10) Different letters in each column indicate significant differences among inoculation conditions for each tissue according to the Duncan's test (P < 0,05). Nd: not determined.

inoculated with *Bradyrhizobium* sp. C-145 was higher than that found in other inoculation conditions. On the other hand, roots and nodules of plants inoculated with *Bradyrhizobium* sp. SEMIA6144 presented the highest As content. Interestingly, inoculated plants revealed higher As content compared to non-inoculated plants in below-ground organs. Regarding As translocation from roots to shoots, inoculation of peanut plants with *Bradyrhizobium* sp. SEMIA6144 revealed a significant lower TF among inoculation conditions proved (Table 2).

In inoculated maize plants, metalloid accumulation was mainly detected in roots compared with shoots. On the contrary, non-inoculated plants accumulated higher As content on shoot than roots. The comparison between inoculated strains revealed that shoot and root As content of plants inoculated with *A. brasilense* CD was higher than that found with *A. brasilense* Az39. As observed in peanut plants, inoculated maize plants presented higher As content in roots compared to non-inoculated plants. Regarding As translocation from roots to shoots, inoculation of maize plants decreased metalloid TF in a significant way compared to non-inoculated plants. Metalloid distribution pattern in the studied crops are in agreement with that found in non-hyperaccumulating legumes (Bianucci *et al.*, 2017, 2018; Mandal *et al.*, 2008) and in poaceae (Derlicková *et al.*, 2013). Taking into account the results presented, *Bradyrhizobium* sp. SEMIA6144 could constitute a promising inoculant for peanut plant in order to decrease As translocation to shoots avoiding fruit contamination. Although, maize inoculation with both tested PGPB avoid metalloid translocation to aerial part of the plant, *A. brasilense* Az39 also promoted growth. Thus, it is possible to suggest that the selection of the best PGPB-plant interaction that not only improve growth but also reduce As accumulation in shoots and therefore in the harvest product, could be a promising biotechnological tool to be used in contaminated field.

4 CONCLUSIONS

Arsenic negatively impacts on peanut and maize growth and the inoculation with PGPB represents

an effective and promising strategy to improve plant development by restraining metalloid translocation to edible parts. In this sense, the rhizoinoculation with *Bradyrhizobium* sp. SEMIA6144 in peanut plants and *Azospirillum* Az39 in maize, results a biotechnological approach to prevent metalloid translocation avoiding As distribution in the trophic chain.

Thus to prepare a strategy for safe tubewell installation leading to the development of a method combining hydrogeological suitability and social mapping which allowed to optimize the locations for safe well installations.

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