

EFFECT OF ARSENIC ON DRY WEIGHT AND RELATIVE CHLOROPHYLL CONTENT IN GREENING MAIZE AND SUNFLOWER TISSUES

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ABSTRACT

Arsenic is one of the most toxic elements that can be found in the environment. Excessive uptake of arsenic may cause physiological changes in plants. The aim of the study was to investigate the effect of different arsenic treatments on relative chlorophyll content and dry weight of shoot and root of maize and sunflower in the early phases of plant development. Seedlings were grown in climatic room in nutrient solution under strictly regulated environmental conditions. The plants were exposed to 3, 10 and 30 mg kg⁻¹ arsenic, whereas there was no arsenic treatment on the control plants. We applied arsenic in the form of arsenite (NaAsO₂) and arsenate (KH₂AsO₄), respectively. After 14 days of arsenic treatments, changes in relative chlorophyll content and dry weight of maize shoots and roots were recorded. In the case of sunflower these physiological parameters were measured after 21 day. The applied arsenic decreased the relative chlorophyll content of maize and sunflower leaves, especially at concentration of 30 mg kg⁻¹. The increasing amount of As treatment were resulted the lower weight of the experimental plants, which was more considerable in the case of the roots. The results indicate that the sunflower plants is more sensitive to arsenic toxicity than maize plants and all data demonstrate that the As(III) is more toxic to these crop plants than the As(V).

Keywords: Arsenic, maize seedling, sunflower seedling, dry weight, relative chlorophyll content

INTRODUCTION

Arsenic (As) is an environmental toxin that is found naturally in soils all over the world (Smedley and Kinniburgh, 2002). The sources of arsenic pollution are mining activities, use of arsenical herbicides and insecticides, irrigation with arsenic contaminated groundwater and some other agricultural and anthropogenic factors (Stoeva et al., 2003; Zhao et al., 2008). Arsenic contamination of soil and groundwater is a global environmental problem affecting a large number of populations (Shaofen Wang et al., 2004). Arsenic becomes part of the food chain when crops and fodder become contaminated (M. Finnegan et al., 2012). Arsenic is nonessential and toxic to plants (Kim et al., 2012). Arsenic phytotoxicity depends on the form and availability of arsenic. Inorganic arsenic compounds are more toxic than organic compounds (Adriano, 1986). Arsenic in soil and in groundwater mostly found as trivalent arsenite (As(III)) or pentavalent arsenate (As(V)). Both are toxic, however, arsenite is the more toxic form, because able to react with sulfhydryl groups of enzymes inhibit the cellular function. Cellular membranes become damaged in plants, which causing electrolyte leakage (M. Finnegan et al., 2012). Arsenic is chemically very similar to phosphorus, an essential plant nutrient, which is necessary for plant growth. The behavior of arsenate seems analogous to phosphate. Phosphorus and arsenic share the same transport pathway in higher plant, so the plant also takes in arsenic from the environment, and it causes inhibition of shoot and root growth, reduction of chlorophylls and germination, lead to low grain yield and finally death.

Roots are usually the first tissue to be exposed to As (M. Finnegan et al., 2012). The root system is the primary site of damages when the amount of arsenic reaches the level of toxicity (O'Neill, 1995). When As is absorbed into plant

roots in the form of arsenate, it is reduced to arsenite (Kim et al., 2008). Roots accumulated higher concentration than the shoot. Uptake of arsenic by the cell of the plant roots increasing with increasing arsenic concentration (Porter and Peterson, 1975).

Some studies have reported photosynthetic pigments were also negatively affected by As (Farnese et al., 2014; Leão et al., 2013). The weight decrease in plant may be related with the decrease in the photosynthetic process and in the photosynthetic (Mascher et al., 2002).

The objective of our study was to investigate the effect of arsenic on relative chlorophyll content and dry weight of shoot and root of maize and sunflower in the early growth phases.

MATERIAL AND METHODS

Plants and growing conditions

To arrange germination, the sunflower seeds were sterilized with 10% H₂O₂ for 10 min and washing in distilled water, in the case of maize after that the seeds soaked in 0.2 μM CuSO₄.

Seeds of maize and sunflower were germinated between moist filter paper and after 3 days seedlings were transported into 1.7 dm³ plastic pots containing an aerated nutrient solution of 2.0 mM Ca(NO₃)₂, 0.7 mM K₂SO₄, 0.5 mM MgSO₄, 0.1 mM KH₂PO₄, 0.1 mM KCl, 1 μM MnSO₄, 1 μM ZnSO₄, 0.2 μM CuSO₄, 0.01 μM (NH₄)₆Mo₇O₂₄, 10⁻⁴ M Fe-EDTA (Cakmak and Marschner, 1990). Nutrient solution contained 0.1 μM H₃BO₃ in the case of maize and 10 μM H₃BO₃ if the plant was sunflower. The nutrient solution were replaced twice a week.

They were grown in climatic room under strictly controlled conditions. Irradiance of 300 mol m⁻² s⁻¹, 16-h day and 8-h night photoperiod, day/night temperature of 25/20°C and relative humidity of 65-75%.

Maize and sunflower seedlings were exposed to 0, 3, 10 and 30 mg kg⁻¹ arsenic. We applied arsenic in two inorganic forms, namely arsenite (NaAsO₂) and arsenate (KH₂AsO₄). The plants were treated separately with As(III) and As(V). At the end of the experimental period the tissues from all the plants were separated into the following parts: roots and shoots.

The dry weight (DW) of shoots and roots were measured after 14 days in the case of maize, and after 21 days in the case of sunflower. The dry weights were recorded after drying the shoots and roots at 65°C.

Relative chlorophyll content (RCC) was recorded on the final day of the experiment. RCC was obtained by using SPAD 502 (Soil Plant Analysis System) chlorophyll meter.

Chemicals were obtained from Sigma-Aldrich Ltd. (Poole, UK) and Chlorophyll meter (Minolta SPAD-502) was supplied by Konica-Minolta, Japan.

Data analysis

All data were subjected to one-way analysis of variance (ANOVA) and Duncan's test at 0.05 significance level, performed on SPSS statistics software version 22.

RESULTS AND DISCUSSION

Effect of arsenic on dry weight of maize seedling tissues

The effect of As (V) treatments on the shoots dry weight and roots dry weight of maize after 14 days is demonstrate in the table 1.

Negative correlation was observed between the higher concentration of As(V) in the nutrition solution and the dry weight of the maize shoots and roots.

In maize, the shoots dry weight decreased by 57-76% in plants which treated with arsenate 3-30 mg kg⁻¹, the roots dry weight decreased by 9-56%.

Table 1 The effect of As (V) treatments (mg.kg⁻¹) on dry weight (DW) (g.plant⁻¹) of maize seedlings roots and shoots. n=4±s.e.

As-treatments (mg.kg ⁻¹)	DW of shoots	DW of roots
0	0.1462±0.0166 ^c	0.0456±0.0095 ^b
3	0.0624±0.0088 ^b	0.0413±0.0051 ^b
10	0.0432±0.0182 ^{ab}	0.0293±0.0117 ^{ab}
30	0.0344±0.0028 ^a	0.0200±0.0005 ^a

DW-dry weight

Dry mass of shoots and roots of maize plants decreased as a result of higher concentration of As(III) in the nutrient solution (table 2.).

Table 2 The effect of As(III) treatments (mg.kg⁻¹) on dry weight (DW) (g.plant⁻¹) of maize seedlings roots and shoots. n=4±s.e.

As-treatments (mg kg ⁻¹)	DW of shoots	DW of roots
0	0.1462±0.0166 ^c	0.0456±0.0095 ^b
3	0.0700±0.0246 ^b	0.0356±0.0085 ^b
10	0.0328±0.0032 ^a	0.0280±0.0014 ^{ab}
30	0.0157±0.0051 ^a	0.0130±0.0013 ^a

DW-dry weight

The dry weight of the control maize plants shoots were approximately 9 times higher than in the case of 30 mg kg⁻¹ As(III) treatment. The shoots dry weight decreased by 50-78%, if the plants were treated with 3 and 10 mg.kg⁻¹. In the case of roots the results did not show significant difference between the control

and the 3-10 mg.kg⁻¹ treatments. If the nutrient solution contained 30 mg.kg⁻¹ As(III) the dry weight of roots decreased by 71% compared to the control. The result shows the same tendency than Stoeva et al. (2003) found in their research about the maize.

Effect of arsenic on dry weight of sunflower seedling tissues

The presence of As(V) in the nutrient solution caused an reduction in the weight of sunflower seedlings (table 3.).

Table 3 The effect of As (V) treatments (mg.kg⁻¹) on dry weight (DW) (g.plant⁻¹) of sun flower seedlings roots and shoots. n=4±s.e.

As-treatments (mg.kg ⁻¹)	DW of shoots	DW of roots
0	0.1830±0.0445 ^c	0.0657±0.0221 ^{ab}
3	0.0965±0.0165 ^b	0.0413±0.0338 ^b
10	0.0335±0.0195 ^a	0.0329±0.0212 ^{ab}
30	0.0267±0.0080 ^a	0.0079±0.0013 ^a

DW-dry weight

This values show that increasing amount of As (V) treatment resulted the lower average weight of the experimental plants. Sunflower seedlings that had been subjected to 3 and 10 mg.kg⁻¹ As (V) stress, shoot dry weight were reduced 47-18%. In the case of shoot dry weight there was no significant difference between the 10 and 30 mg.kg⁻¹ treatments. The control plant root dry weight was 1.5 times higher than case of 3 mg.kg⁻¹ and dry mass of roots were less than 0.1g in all cases.

The weight of sunflower shoots and roots decreased with the different concentration of As (III) treatment that have been demonstrated in table 4.

Table 4 The effect of As (III) treatments (mg.kg⁻¹) on dry weight (DW) (g.plant⁻¹) of sunflower seedling roots and shoots. n=4±s.e.

As treatments (mg.kg ⁻¹)	DW of shoots	DW of roots
0	0.1830±0.0445 ^c	0.0457±0.0221 ^a
3	0.0942±0.0092 ^b	0.0494±0.0015 ^a
10	0.0408±0.0020 ^{ab}	0.0214±0.0061 ^a
30	0.0154±0.0019 ^a	0.0117±0.0019 ^a

DW-dry weight

At the end of the 14 days period of arsenic stress, the dry mass of roots decreased 49-92% compared to the control. When we treated the sunflower plants in the early growth phases of plant development with 3 mg.kg⁻¹ arsenite or more, we found that the average dry weight of shoots mass reduced from 0.1830g to less than 0.1 g.

Compared the physiological response of maize and sunflower to the different arsenic treatments, we found that the maize was more tolerant to arsenic exposure than the sunflower.

Effect of arsenic on relative chlorophyll content of maize and sunflower seedlings

The result of our research on relative chlorophyll content of maize and sunflower seedlings are summarized in table 5 and in table 6.

The relative chlorophyll content in the leaves of maize changed to presence of As(III) and As(V) as compared to control. In the case of As(III) treatment the level of relative chlorophyll content at 3 mg.kg⁻¹ and 10 mg.kg⁻¹ increased by 10 and 14%, respectively. The highest As(III) concentration caused increase of relative chlorophyll content by 21%. In the case of As(V) the maize seedlings relative chlorophyll content decrease by 9-18%, if the plant was treated with 3-30 mg kg⁻¹.

Table 5 The effect of As (III) and As (V) treatments (mg.kg⁻¹) on relative chlorophyll content (RCC) of maize seedlings leaves. n=4±s.e.

As-treatments (mg.kg ⁻¹)	RCC (As(V))	RCC (As(III))
0	42.16±0.96 ^c	42.16±0.98 ^d
3	38.26±0.66 ^b	38.10±0.98 ^c
10	36.93±1.60 ^a	36.10±0.40 ^b
30	34.53±0.33 ^a	33.37±0.14 ^a

RCC- relative chlorophyll content

In the case of sunflower the changes in relative chlorophyll content followed the same tendency. The average relative chlorophyll content of sunflower 42.16, when the sunflower was grown in nutrient solution without As treatment. The highest decrease in the relative chlorophyll content observed at concentration of 30 mg.kg⁻¹.

Table 6 The effect of As (III) and As (V) treatments (mg.kg⁻¹) on relative chlorophyll content (RCC) of sunflower seedlings leaves. n=4±s.e.

As-treatments (mg.kg ⁻¹)	RCC (As(V))	RCC (As(III))
0	42.00±0.05 ^a	42.00±0.57 ^c
3	40.40±0.25 ^b	39.53±0.38 ^b
10	38.46±0.46 ^{ab}	36.76±0.29 ^a
30	36.48±0.62 ^c	36.53±0.33 ^a

RCC- relative chlorophyll content

CONCLUSION

This study provides important information concerning the relationship between the endogenous levels of As in plants and its impact on dry weight and relative chlorophyll content.

Result of our experiments shows treatments of the maize and sunflower roots with As had a negative effect on relative chlorophyll content and dry mass of shoots and roots.

We observed that sunflower plants is more sensitive to arsenic toxicity than maize plants and all data demonstrate that As (III) is more toxic to these plants than As (V).

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