C. R. Biologies 333 (2010) 814–824

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Plant biology and pathology/Biologie et pathologie végétales

# Enzymatic adaptations to arsenic-induced oxidative stress in *Zea mays* and genotoxic effect of arsenic in root tips of *Vicia faba* and *Zea mays*

Isabelle Duquesnoy [a,b,c,d], Gabrielle Marie Champeau [a,b], Germaine Evray [a,b], Gérard Ledoigt [a,b], Agnès Piquet-Pissaloux [c,d,*]

[a] *Clermont université, Université Blaise-Pascal, Piaf, BP 10448, 63000 Clermont-Ferrand, France*
[b] *INRA, UMRA 547, Piaf, 63100 Clermont-Ferrand, France*
[c] *Clermont Université, VetAgro Sup, BP 10448, 63000 Clermont-Ferrand, France*
[d] *VetAgro Sup, campus agronomique Clermont, Aquise, BP 35, 63370 Lempdes, France*

ARTICLE INFO

*Article history:*
Received 3 December 2009
Accepted after revision 27 July 2010
Available online 8 October 2010

*Keywords:*
Arsenate
Arsenite
Physiological parameters
SOD
G-POD
APX
CAT
Genotoxic parameters
*Zea mays*
*Vicia faba*

ABSTRACT

Agronomic plant species may display physiological and biochemical responses to oxidative stress caused by heavy metals and metalloids. *Zea mays* plants were grown hydroponically for eight days at different concentrations of As (0, 134 and 668 μM) and at different pH (4, 7 and 9). Metabolic variations in response to As toxicity were measured using physiological parameters and antioxidant enzymatic activities. A significant decrease in SOD activity was observed in the leaves and roots of *Z. mays* with the majority of As treatments. As decreased G-POX activity less in leaves than in roots. An increase in the concentration of As increased APX activity in leaves and roots, except As(V) at pH 4 and pH 9 in the leaves and As(III) at pH 9 in the roots, when there was a significant decrease in APX activity at low As concentrations. After exposure to As(V), CAT activity was the same as in the control. As(III) led to an increase in CAT activity in leaves and to a decrease in roots. With increasing concentrations of As(III), CAT activity increased in both leaves and roots whatever the pH. To obtain more detailed knowledge on the effects of arsenate and arsenite exposure on *Vicia faba* and *Z. mays*, root meristems were also examined. Roots were fed hydroponically with 134, 334, 534 and 668 μM arsenate or arsenite and $4 \times 10^{-3}$ M of maleic hydrazide as positive control, at three different pH. Physiological parameters, the mitotic index and micronuclei frequencies were evaluated in root meristems. At all three pH, the highest As(V) and As(III) concentrations induced a substantial modification in root colour, increased root thickness with stiffening, and reduced root length. High concentrations also caused a significant decrease in the mitotic index, and micronucleus chromosomic aberrations were observed in the root meristems of both species.



## Abreviations

| | |
|---|---|
| As(V) | arsenate |
| As(III) | arsenite |
| HM | maleic hydrazide acid |
| SOD | superoxide dismutase |
| G-POD | guaiacol peroxidase |
| APX | ascorbate peroxidase |
| CAT | catalase |
| MI | mitotic index |
| MN | micronucleus |
| NBT | nitro blue tetrazolium |
| DETAPAC | diethylenetriaminepentaacetic acid |
| FB | fresh biomass |
| PC | protein contents |
| ROS | reactive oxygen species |

* Corresponding author.
*E-mail addresses:* a.piquet@vetagro-sup.fr, piquetagnes@orange.fr (A. Piquet-Pissaloux).

1631-0691/$ – see front matter © 2010 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.crvi.2010.07.004

## 1. Introduction

With the development of modern industry and agriculture, problems due to arsenic pollution are attracting increasing attention. Inorganic arsenic species (As(V) and As(III)) have long been used in the formulation of pesticides and herbicides [1]. The toxic effects of arsenic disrupt crop growth and development [2–4]. This effect varies with the form and concentration of arsenic in the soil, with the tolerance of the genotype species to the toxic element and with the environmental conditions governing growth [3,5–7]. Some authors have reported that metalloids and heavy metals (As, Cd, Pb) pollution induce free radicals that lead to the readjustment of transport and of metabolic processes, inhibit growth [8,9] and may damage major cell macromolecules (proteins, lipids and DNA) [10–12]. However, plants do possess several anti-oxidative defence systems to scavenge toxic free radicals to protect themselves from oxidative stress, including that caused by heavy metals and metalloids [13]. These systems include:

(I) reduced uptake and accumulation of metals by binding the metal to extracellular exudates and cell wall constituents;
(II) compartmentation of metals in the vacuole;
(III) complexation of heavy metals and metalloids by different substances;
(IV) activation or modification of plant metabolism;
(V) production of antioxidant enzymes.

Anti-oxidative defence falls into two main categories:

(I) low molecular mass antioxidants, consisting of lipid soluble membrane-associated antioxidants (e.g. α-tocopherol and β-carotene) and water soluble reductants (e.g. glutathione–GSH and ascorbate);
(II) enzymatic antioxidants (e.g. SOD, CAT, G-POD, APX [14–18]).

Induction of oxiradicals by arsenic may be linked to the mechanisms involved in genotoxicity. Plant bioassays, which are considerably less expensive than animal assays, have been proposed to monitor pollution. Some plant bioassays, i.e. *Tradescantia palludosa* [19–21], *Allium cepa* [22–24] and *Vicia faba* [25], have been used for over 60 years to study the mutagenic effects of ionizing radiation and chemical mutagens, but also more recently to evaluate the mutagenicity/clastogenicity of environmental pollutants. In recent years, a correlation between arsenic exposure, cytotoxicity and genotoxicity, mutagenicity [3], and tumour promotion has been established [26]. The causes of the toxic effects of excessive arsenic have been the subject of many studies [7,9,27]. Trivalent forms of arsenic are more toxic than pentavalent forms [28]. Two arsenic species have been shown to induce apoptosis in several cellular systems [29] and are capable of triggering apoptosis [30]. In addition, arsenic causes induction of chromosome/chromatid breaks or exchanges (clastogenicity) [8], formation of apurinic/apyrimidinic sites [10], DNA and oxidative base damage [12], DNA-protein cross-links [31], chromosomal aberrations and interaction with spindle function during mitosis or meiosis inducing chromosome segregational errors (non-disjunction and non-congression), leading to aneuploidy and/or polyploidy (aneugenicity) [32]. The study of the potential biochemical and genotoxic effects of arsenic on plants (i.e. the first stage in the food chain for humans) must guarantee plant quality. *Zea mays*, a member of the Poaceae family, is one of three most important crop plants in the world. Maize is one of the plant species used for evaluating the potential genotoxicity of environmental chemicals [33,34]. Since many plants are known to be injured by arsenic contamination [1,35], some plant systems have been suggested as indicators of arsenic exposure [10,36]. In this study, to evaluate biochemical and genotoxic responses to different types of As pollution, *Z. mays* was grown hydroponically for eight days with different As treatments: arsenate As(V) and arsenite As(III) at 134 and 668 μM, at pH 4, 7 and 9. The effects of As on the growth and activity of the enzymatic antioxidant system (SOD, APX, G-POD, CAT) in the leaves and roots were studied. To understand the toxic effects of arsenic on plant cells in more detail, mitotic activity was investigated in *Z. mays* and *V. faba* roots by monitoring the MI. In addition, MN assays were performed on root tips of *Z. mays* and *V. faba*. The results obtained should lead to a better understanding of the adaptation mechanisms of this agronomic species to As and of the mechanism of cytological damage.

## 2. Materials and methods

### 2.1. Plant material, growth conditions and treatment procedures

*Z. mays* seeds were obtained from Limagrain in Auvergne, France. The seeds were rinsed with distilled water and transplanted in 1.2-litre plastic (PVC) pots for 1 month. The plants were maintained in a Hoagland medium [37] comprising $KNO_3$: 101.1 mg $L^{-1}$; $KH_2PO_4$: 236.5 mg $L^{-1}$; $CaNO_3$, $4H_2O$: 54.44 mg $L^{-1}$; $MgSO_4$, $7H_2O$: 73.94 mg $L^{-1}$; $MnSO_4$, $H_2O$: 1.5 mg $L^{-1}$; $ZnSO_4$, $7H_2O$: 0.5 mg $L^{-1}$; $CuSO_4$, $4H_2O$: 0.25 mg $L^{-1}$; $H_3BO_3$, $4H_2O$: 1.5 mg $L^{-1}$; $Na_2MoO_4$, $2H_2O$: 0.025 mg $L^{-1}$ and 100 μL $L^{-1}$ Fe-masqualate. Before the medium was autoclaved, its pH was adjusted to respectively 4, 7 and 9 [33]. The solutions were placed in a controlled environment room at 25 ± 1 °C with a photoperiod of 16 h/8 h light/dark. For the experiments, 10 plants, replicated three times, were taken from the stock cultures and exposed to different concentrations of arsenate and arsenite (134 and 668 μM) at three different pH for eight days, according to the procedure described in Tu et al. [34]. Briefly, arsenate ($As_2O_5$, 99% purity, Sigma, France) and arsenite ($AsO_3$, 99.99% purity, Sigma, France) were dissolved in growth medium (1 l of Hoagland medium) and the pH was adjusted with NaOH (10 M) or KCl (1 M).

### 2.2. Biomass determination

After arsenate and arsenite treatments, plants were washed three times with distilled water and the fresh biomass of leaves and roots was determined.