Arsenate toxicity on the apices of Pisum sativum L. seedling roots: effects on mitotic activity, chromatin integrity and microtubules

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Stefania Dho, Wanda Camusso, Marco Mucciarelli, Anna Fusconi

Abstract

Arsenic (As) is one of the most toxic pollutants in the environment, where it severely affects both animal and plant growth. Despite the growing literature data on As effects on plant development, alterations induced by this element on meristem activity of the root have not been explored to any great extent. In the present study, short-term experiments with arsenate have been conducted on *Pisum sativum* L. seedlings to assess whether plant growth impairment is due to DNA/chromosome or mitotic microtubule damages. Root growth was studied by evaluating apical meristem activity and cell elongation. Mitotic aberrations, DNA fragmentation and microtubule organization of the apical cells were also analyzed. The results have shown that arsenate, at the lowest concentration (0.25 μM), slightly increases root growth and some related parameters, whilst the other concentrations have a dose-dependent negative effect on root growth, on the mitotic and labelling index (after bromo-deoxyuridine administration), and on the mitotic arrays of microtubule (through immunofluorescence). The main effects on mitosis occurred for 25 μM As. The percentage of metaphases increased, as did the irregular metaphases and c-mitoses. This was related to alterations in the mitotic spindles, which closely resemble those induced by colchicine. Chromosome breaks and ana/telophase bridges were virtually absent, whilst DNA fragmentation only increased from 25 μM arsenate onwards. These data point to a poor clastogenetic activity of As and implicate that microtubules are one of the main targets of As.

Keywords

Pea; Arsenic; Apical meristems; Aberrations; Immunofluorescence; TUNEL test

1. Introduction

Arsenic (As) is a toxic element, frequently found in soils and water. A main natural source of As is the erosion of mother rock, even though a consistent part of As environmental pollution comes from human activities (Meharg and Hartley-Whitaker, 2002 and Patra et al., 2004). The As in unpolluted fresh water is usually in the range 1–10 μg/l. According to EPA and WHO, the maximum permissible As concentration in drinking water is 50 μg/l (Mandal and Suzuki, 2002).

Arsenic is a well-established human carcinogen (Qin et al., 2008a) and has been shown to be genotoxic in a variety of *in vitro* studies (Hughes, 2002). In plants, it severely affects growth and development, and its toxicity is strongly dependent on the concentration, exposure time and physiological state of the plant (Singh et al., 2007). However, plants vary in their sensitivity to As, and a wide range of species have been identified in As-contaminated soils (Meharg and Hartley-Whitaker, 2002). Besides, hyperaccumulators such as *Pteris vittata*, which tolerate high internal As content, may also use this As to defence themselves against herbivore attack (Mathews et al., 2009).

Higher plants take up As mainly as arsenate (V), the dominant form of phytoavailable As in aerobic soils. According to Meharg and Hartley-Whitaker (2002), As competes with phosphate for plant phosphate transporters. Upon absorption, most arsenate is rapidly reduced to arsenite (III), due to an arsenate reductase activity (Xu et al., 2007), hence, the arsenate cytoplasmic concentration is generally not high enough to exert toxicity (Meharg and Hartley-Whitaker, 2002). Both As species interfere with various metabolic pathways: arsenate, as an analogous chemical to phosphate, may replace phosphate in the ATP and in various

phosphorylation reactions, leading to the disruption of the energy flow in cells. The toxicity of arsenite is mainly ascribed to its reaction with sulphydril groups of proteins that interfere with their functions (Meharg and Hartley-Whitaker, 2002 and Patra et al., 2004).

Exposure to high concentrations of As induces the production of reactive oxygen species (ROS) (Singh et al., 2007, Wang et al., 2007, Lin et al., 2008 and Shri et al., 2009) and the conversion of arsenate to arsenite is regarded as one of the causes of ROS generation (Wang et al., 2007). Oxidative stress induced by As can damage cells, mainly through lipid peroxidation of membranes (Singh et al., 2007) and DNA fragmentation, as has been demonstrated in leaves and roots of *Vicia faba* using the single-cell gel electrophoresis method (Lin et al., 2008). DNA damage is further increased by As inhibition of enzymes involved in DNA repair (Yi et al., 2007), and this occurs even at low As concentrations, in human cultured cells (<1 μM, Qin et al., 2008a).

Although the biochemical and physiological disorders that lead to growth inhibition of the root and of the whole plant are relatively well known (Liu et al., 2005, Singh et al., 2007, Abercrombie et al., 2008 and Lin et al., 2008), the alterations of meristem activity and cell elongation, which represent the direct cause of growth inhibition, have not been explored to any great extent in plants. Moreover, As is generally considered an aneuploidy-inducing agent due to its interactions with the spindle function (Panda and Panda, 2002), but to the authors' knowledge, there is a lack of data about microtubule (MT) alterations caused by As in plants.

The aim of the present paper was to verify As effects on root growth by measuring the length of fully differentiated cells and to evaluate apical meristem activity through analysis of the percentage of mitosis and DNA synthesizing cells. In order to assess whether growth inhibition is related to DNA/chromosome damage or mitotic microtubule impairment, we quantified the occurrence of mitotic aberrations and DNA fragmentation through the TUNEL test, and also visualized the MT mitotic arrays through immunofluorescence. We administrated As in the form of arsenate, the molecular species generally found in aerobic soils, and used *Pisum sativum* seedlings; this is an As-sensitive species (Päivöke and Simola, 2001) widely used to study stressing factors, such as heavy metals and irradiance (see, for example, Zaka et al., 2002, Adamakis et al., 2008, Adamakis et al., 2010 and Békésiová et al., 2008).

2. Materials and methods

2.1. Plant material, growth conditions and morphometry

Seeds of *P. sativum* L. cv. Meraviglia d'Italia (Fratelli Ingegnoli, Milan, Italy) were surface-sterilized with sodium hypochlorite (0.6% active chlorine) for 10 min, rinsed, and germinated on moistened cotton wool, in the dark at 25 °C. Three days after sowing, the seedlings were transferred, for 24 h, into vials containing an aerated 0.5 mM calcium sulphate solution, pH 6.0. A simple calcium solution was used because the first stages of seedling growth are completely sustained by seed reserves. Only seedlings with well growing roots were transferred to the calcium sulphate solutions containing 0, 0.25, 2.5, 25 and 250 μM of arsenate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma–Aldrich, Milan, Italy), pH 6.0, for an additional 24 h.

Primary roots of 10 seedlings per treatment were measured before and after exposure to As to assess root lengthening. Root lengthening was determined on each group of seedlings, before proceeding with further experiments, to verify the effect of As concentrations on growth. Cell lengths of 100 cortex cells per treatment were measured, at an ocular micrometer scale, on free hand longitudinal sections taken at a distance from the root apices corresponding to the last 24 h of lengthening.

2.2. Meristem activity and mitotic aberrations

The primary roots of 10 seedlings per treatment were fixed in 3:1 (v:v) ethyl alcohol/acetic acid for 1 h at room temperature. Mitotic activities were evaluated on squashes of Feulgen stained root apices. At least 1000 cells per tip were scored. The mitotic index (MI, i.e. the % of mitotic cells) and the mitotic phase index (the number of cells in each mitotic phase expressed as the percentage of the total mitotic cells) were calculated on the same slide, as was the percentage of aberrant mitoses.

Three seedlings per treatment were fed for 1 h with a 50 μM 5-bromo-2′-deoxyuridine (BrdU) solution, containing 5 μM of 5-fluoro-2′-deoxyuridine (Amersham International, Buckinghamshire, UK), to determine the labelling

index (LI). Nuclei were fixed and extracted, as described by Fusconi et al. (2007). BrdU was detected with a nuclease/mouse anti-BrdU (Amersham International, Buckinghamshire, UK), overnight at 4 °C, followed by an anti-mouse IgG/Cy3 conjugated (Sigma–Aldrich, Milan, Italy) secondary antibody and counterstained with 0.2 µg/ml DAPI (4′,6-diamidino-2-phenil-indole, Sigma–Aldrich, Milan, Italy). The LI was determined as the percentage of BrdU labelled nuclei in relation to the DAPI stained nuclei, by counting about 1000 nuclei per treatment, and the entire procedure was repeated three times. The stained nuclei were observed using a Nikon Eclipse E400 fluorescence microscope, with G-2A (Cy3) and UV 2A (DAPI) filters and images were acquired with a Nikon Ds-5M digital camera.

2.3. TUNEL test

The nuclei of three root apices per treatment were extracted and spread on microscope slides, as previously described. DNA strand breaks were detected and visualized using the *In Situ* Cell Death Detection Kit-Fluorescein (Roche, Penzberg, Germany), according to the procedure recommended by the manufacturer. The slides were counterstained with 0.2 µg/ml DAPI, observed by fluorescent microscopy as previously described (filter B-2A for fluorescein), and the percentages of labelled nuclei were calculated. The test was repeated three times for each treatment.

2.4. Microtubule (MT) morphology of mitotic cells

Five root apices per treatment were processed for indirect immunofluorescence, according to the usual techniques (Fusconi et al., 2007). A mouse anti-β-tubulin monoclonal antibody (Sigma–Aldrich, Milan, Italy) was used as a primary antibody, followed by an anti-mouse IgG/Cy3 conjugated (Sigma–Aldrich, Milan, Italy). Samples were counterstained with DAPI and observed by fluorescence microscope, as previously described.

2.5. Statistics

The results were expressed as means ± SE and compared by analysis of variance (ANOVA), using Systat 11 software for Windows. Differences were considered as statistically significant at $P < 0.05$ (Tukey–Kramer post hoc test).

3. Results

The root lengthening, MI and LI (calculated after 1 h of BrdU incorporation) in pea roots followed similar trends: all parameters increased at the lowest As concentration (0.25 µM As) and dropped progressively with increasing As (Fig. 1A–C). The root lengthening, MI and LI were approximately halved, compared to the controls, by 25 µM As. Cell length decreased significantly from 2.5 µM As and at 250 µM it was about 40% of the controls (Fig. 1D). Due to the very low number of mitotic cells at 250 µM As, the latter was no longer considered.

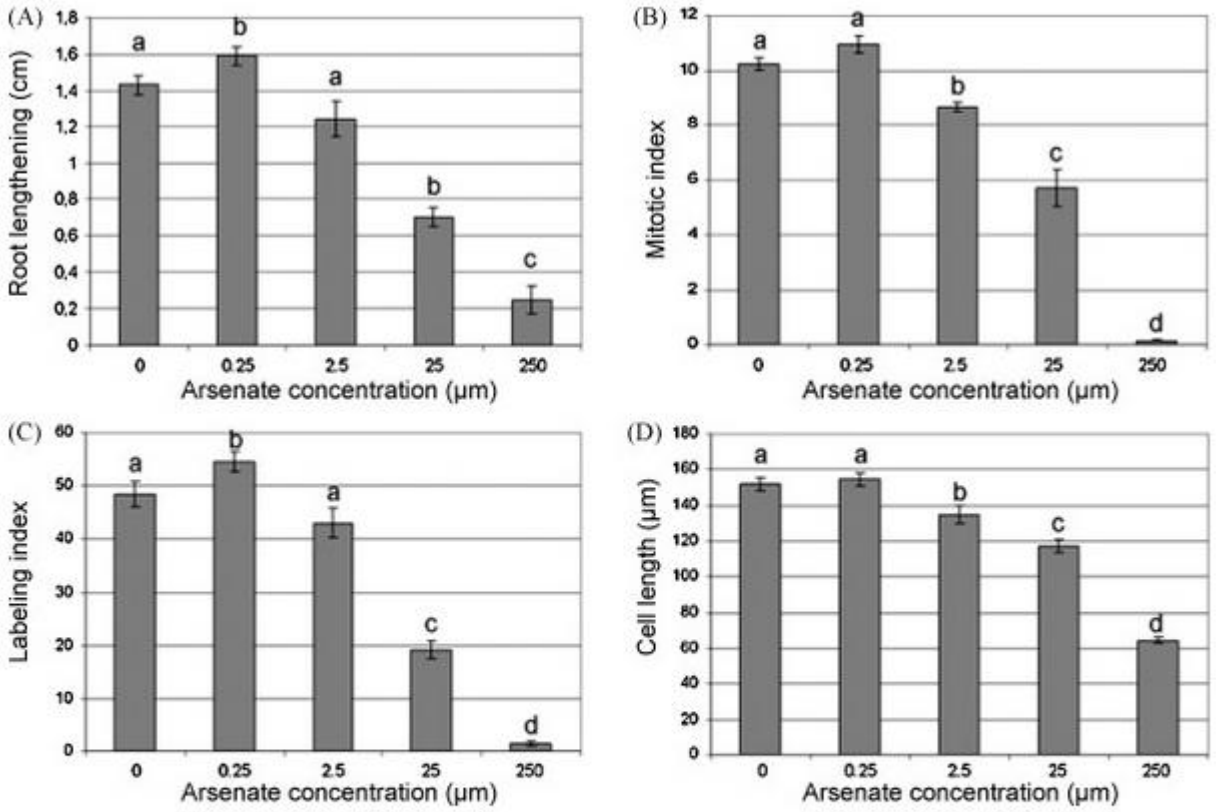

Fig. 1.

Effects of 0.25, 2.5, 25 and 250 µM arsenate on the roots of *Pisum sativum* seedlings after 24 h of exposure. (A) Primary root growth; (B) mitotic index of the root apex; (C) labelling index (percentage of meristematic apical cells labelled for 1 h with BrdU), and (D) cell length in the youngest differentiated zone. All values are represented by means ± SE. Different letters indicate significant differences ($P < 0.05$).

Arsenic affected the percentages of mitotic phases at the 25 µM concentration. In the controls, as well as with 0.25 and 2.5 µM As, the metaphases represented roughly half of the prophases, whilst 25 µM As significantly decreased the percentages of the prophases and increased those of the metaphases, therefore the frequency of the two phases was similar (Fig. 2).

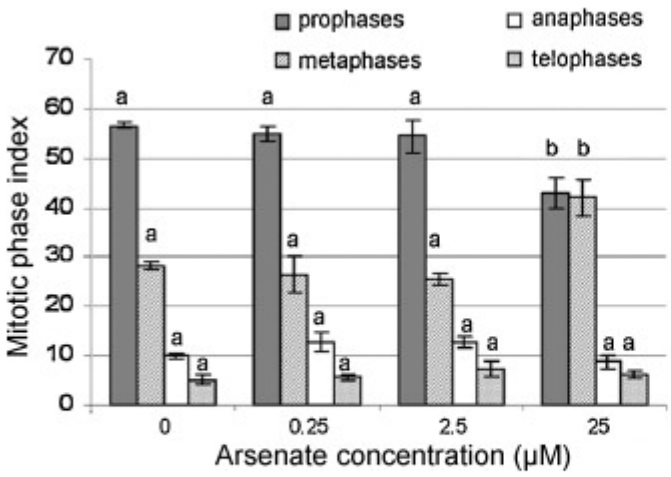

Fig. 2.

Mitotic phase distribution in the apical meristems of *P. sativum* roots after 24 h of treatment with 0.25, 2.5, 25 and 250 μM arsenate. The percentage of each mitotic phase is calculated relative to the mitotic cells. All values are represented by means ± SE. Different letters indicate significant differences ($P < 0.05$).

A lower As administration (0.25 and 2.5 μM) induced about 5% of mitotic aberrations, which increased to about 17% of mitotic cells in the 25 μM As treatment (Fig. 3A). The mitotic aberrations were mainly represented (79.53 ± 5.48% of mitotic aberrations) by irregular metaphases and c-mitoses (colchicine-mitoses), consisting of swollen chromosomes (Fig. 3A, inset). Interphases with dispersed and pale chromatin and pycnotic nuclei were frequent at this concentration (Fig. 4B′). Chromosome fragmentations and ana/telophase bridges were rarely found, whilst a very small number of micronuclei occurred in some root apices of all the treatments (data not shown).

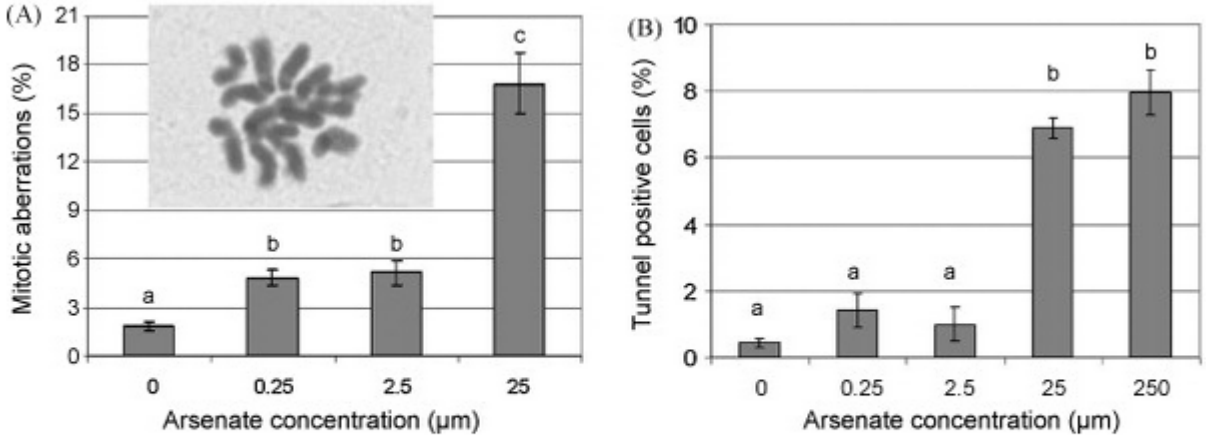

Fig. 3.

Mitotic aberrations (A) and TUNEL positive nuclei (B) percentages in meristematic cells of *P. sativum* roots, after 24 h of arsenate administration. The mitotic aberrations mainly consisted of irregular metaphases and c-mitoses (A, inset). The TUNEL test was conducted on nuclei extracted from *P. sativum* tips, to evaluate DNA fragmentation. Values are represented by means ± SE. Different letters indicate significant differences ($P < 0.05$).

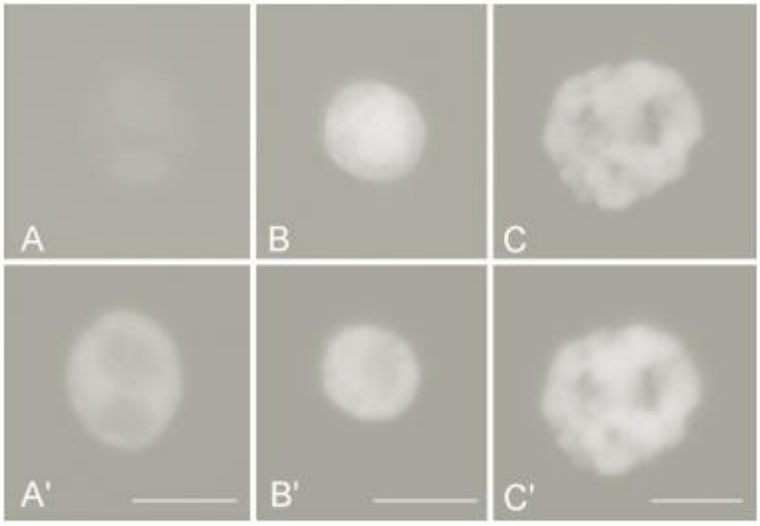

Fig. 4.

Representative images of TUNEL stained nuclei extracted from *P. sativum* tips after 25 μM As exposure: (A) TUNEL negative nucleus, (B) TUNEL positive pycnotic nucleus, and (C) TUNEL positive prophase nucleus. (A', B' and C') Represent the same nuclei after DAPI staining. Bars = 10 μm.

The TUNEL test showed a consistent DNA fragmentation in the interphase and prophase cells after the treatments with 25 and 250 μM As (Fig. 3 and Fig. 4).

Arsenic affected the microtubule (MT) architecture, and the percentage of cells with disturbed MT arrays increased with As concentration in all the mitotic phases (Table 1). Prophase cells with altered preprophase bands (PPBs) and metaphase spindles represented the most frequent kind of alteration, with respect to the controls (Fig. 5 and Fig. 6). Preprophase bands were sometimes absent, even in cells with well condensed prophase nuclei or were sometimes misplaced (Fig. 5B and B′) or broadened. Elongated cells frequently showed irregularly bundled (Fig. 5C and C′) or double (Fig. 5D and D′) PPBs. Alterations in the metaphase arrays of MTs were represented by irregular spindles, formed of thick bundles of MTs (Fig. 6B–B″). In cells showing an irregular chromosome distribution, the spindles often consisted of irregularly thickened bundles of MTs (Fig. 6C–C″). In c-mitoses, the spindles degenerated and appeared as round structures and strands from a front view (Fig. 6D–D″).

Table 1.

Percentage of altered microtubule arrays in the mitotic cells of the apical meristems of *Pisum sativum* roots after 24 h of arsenate exposure.

As concentration (μM)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
0	3.53 ± 0.73 a	5.32 ± 0.32 a	–	–
0.25	16.25 ± 3.75 b	6.25 ± 2.15 a	8.33 ± 3.22 a	–
2.5	50.04 ± 1.44 c	22.78 ± 6.47 b	17.59 ± 4.50 a	11.79 ± 0.69 a
25	65.59 ± 6.08 d	63.52 ± 5.26 c	30.17 ± 2.25 b	22.09 ± 4.08 b

Statistic analysis was performed for each mitotic phase separately. All values are represented by means ± SE. Different letters indicate significant differences in each column ($P < 0.05$).

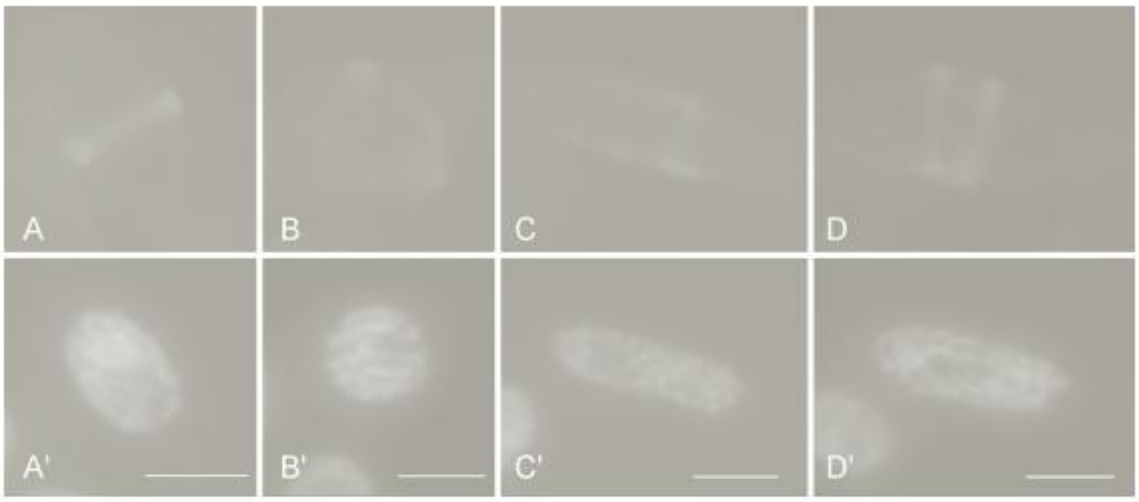

Fig. 5.

Representative images showing the effects of 24 h of exposure to 25 μM arsenate on the preprophasic band (PPB) of the apical cells of *P. sativum* roots. (A–D) Immunofluorescent labelled microtubules and (A'–D') corresponding DAPI stained nuclei. (A and A') Normal, unaffected PPB; (B and B') misplaced PPB; (C and C') irregularly bundled PPB; (D and D') double PPB in an elongated cell. Bars = 10 μm.

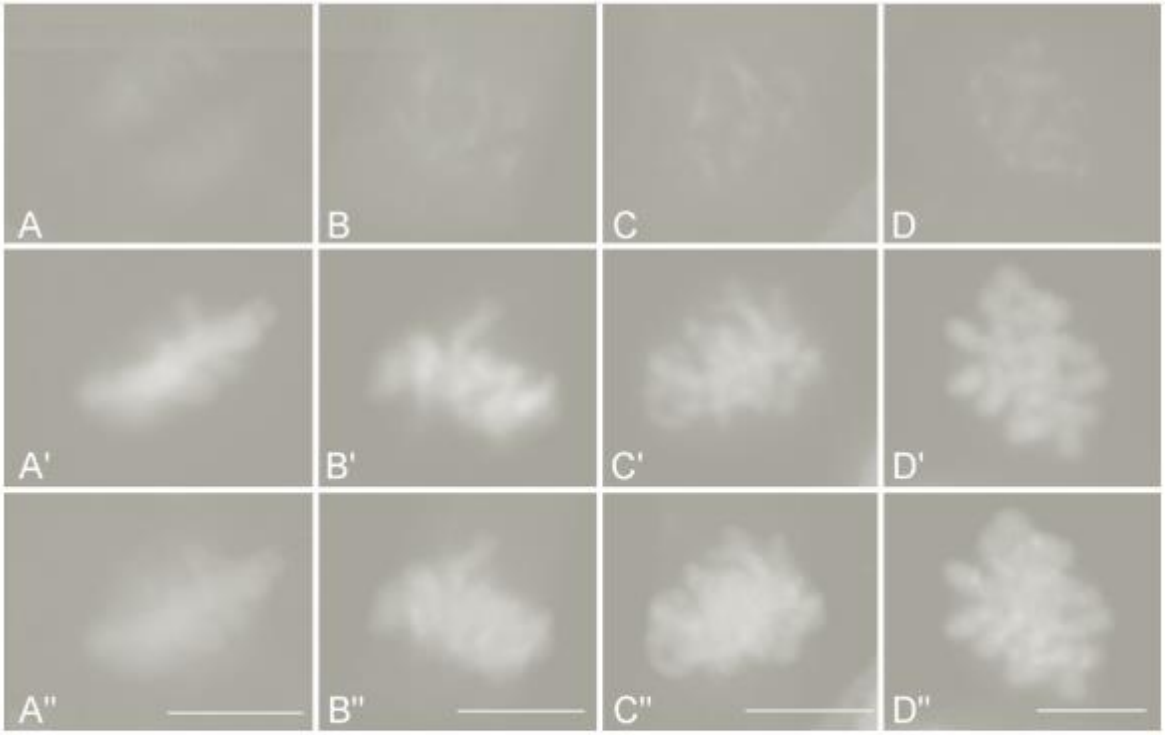

Fig. 6.

Representative images showing the effects of 24 h of exposure to 25 μM arsenate on the mitotic spindle of metaphase cells of the apex of *P. sativum* roots. (A–D) Immunofluorescent labelled microtubules, (A'–D') DAPI stained nuclei, (A''–D'') superimposed images of immunofluorescent labelled microtubules and DAPI stained nuclei. (A–A'') Normal, unaffected cell; (B–B'') cell with an irregular spindle, formed of thick bundles of MTs; (C–C'') cell with no precisely orientated chromosomes and arrowhead-like bundles of microtubules; (D–D'') c-mitosis with remnants of microtubules consisting of round structures and connecting strands. Bar = 10 μm.

4. Discussion

Root lengthening is controlled by the cell division rate in the apical meristems and by expansion and elongation of the newly formed cells. It is considered to be one of the most sensitive endpoints of plant toxicity and a dose-dependent inhibition of root growth (and of the whole plant), following the administration of relatively high doses of arsenate, has been reported for wheat (Liu et al., 2005), mung bean (Singh et al., 2007), *Arabidopsis thaliana* (Abercrombie et al., 2008), broad bean (Lin et al., 2008) and rice (Shri et al., 2009). In the present work, however, the root length and LI, which reflect the cell fraction that passes through the S-phase during the pulse and include the nuclei in the S-phase and part of those in G2 (Fusconi et al., 2007), showed a pleiotropic response to As.

Although a growth stimulation of roots and shoots with low As concentrations has already been reported in the literature following both arsenite and arsenate administration (Mascher et al., 2002, Li et al., 2007 and Shaibur and Kaway, 2009), no explanation for this effect is available concerning plants. One possible explanation could come from animal/human systems where low As levels can act as a carcinogen by enhancing cell proliferation, whilst high levels are chemotherapeutic, inducing cell cycle arrest and apoptosis (Lau et al., 2004). In human epidermal keratinocytes, it has been demonstrated that a low arsenite concentration (0.4 μM) induces a persistent upregulation of D-type cyclins, leading to the abrogation of the cell cycle checkpoint at the G1/S interface, and to the premature transition into the S-phase (Hwang et al., 2006). In pea meristems, under low As levels, only a small number of cells presented cytological alterations (MT damage, DNA fragmentation and chromosomal aberrations) which may block the cell cycle (Reichheld et al., 1999). Since arsenate is rapidly converted to arsenite in plant cells (Meharg and Hartley-Whitaker, 2002), in our experimental system a mechanism responsible for the increased transition from G1 to S, similar to that proposed for arsenite in human keratinocytes, may promote cell production and root lengthening at the lowest As concentration.

Starting from 2.5 μM As, an increasing number of cells exit the cell cycle, as indicated by the reduction in the MI and LI; these values dropped to near zero after the 250 μM treatment. A reduction in meristem activity is a general response to stress and it has been demonstrated that cells respond to oxidative stress by arresting cell division and delaying transition from G1 to S and from G2 to M, or inhibiting DNA replication (Reichheld et al., 1999). Thus, the progressive reduction in the meristem activity, along with the occurrence of shorter cortex root cells, explain the decreased root length at increasing As concentrations. A reduction in MI has been observed in *Allium cepa* and *Hordeum vulgare* plants exposed to As (Patra et al., 2004 and references therein), whilst, to the authors knowledge, no data about the entry of the cell cycle into the S-phase have been reported in the literature for plants.

Furthermore, 25 μM As modified the percentages of the mitotic phases, by blocking the cell cycle at metaphase, as a possible consequence of aberrant metaphases with an irregular distribution of chromosomes and c-mitoses. The occurrence of altered metaphases and lack of chromosome fragmentations, bridges and micronuclei in root apices of *P. sativum* are indicative of a scarce clastogenic activity of As. This is in agreement with our TUNEL results which showed a significant increase in DNA strand breaks from 25 μM As. Even in human keratinocyte cells, low concentrations of As (≤2 μM) alone do not induce significant DNA strand breaks, and As is hence frequently regarded as a co-carcinogenic (Qin et al., 2008b). The DNA degradation observed in the present study was not related to internucleosomal fragmentation, as can be seen by the lack of DNA laddering in the gel electrophoresis preparations (not shown), therefore it is probably not related to programmed cell death, as occurs, for example, after cadmium administration (Behboodi and Samadi, 2004).

Our data have demonstrated that As causes a range of alterations of the MT arrays, in a dose-dependent manner and the highest As concentration (250 μM) leads to an almost complete loss of MTs.

Arsenic affects the preprophasic bands (PPBs), which doubled, became misplaced or even irregularly bundled. Double PPBs do not represent an abnormality *per se*, since they occur, at a very low frequency, in untreated mitotic cells of *P. sativum*, as well as in other species (Galatis et al., 1983 and Utrilla et al., 1993). However, they greatly increased after As administration. The correct development of the PPB at the transition from interphase to mitosis is based on the dynamic instability of the MTs (Dhonukshe and Gadella, 2003) which can be suppressed

by MT-targeted agents (Bhattacharyya et al., 2008). Malfunctioning of MT-stabilizing or destabilizing proteins (Dhonukshe and Gadella, 2003), alterations of the actin microfilaments (Eleftheriou and Palevitz, 1992), as well as the reduction of the DNA replication rate (Giménez-Abián et al., 2004), may be involved in the induction of PPB alterations.

A regular PPBs is probably not necessary for the sequence of mitotic events, since it has been demonstrated that spindles and phragmoplasts are formed in cells in which the PPBs have been experimentally removed and in mutants that affect PPB formation (Marcus et al., 2005). On the contrary, damage of the mitotic spindle and the consequent formation of c-mitosis, cause a mitotic block which, in the present work, is reflected by the increase in metaphase cells, following administration of 25 µM As.

In *P. sativum* root apices, spindle damage closely resembled that induced by colchicine in root meristems of *A. cepa* (Utrilla et al., 1989). In this species, 1 mM colchicine leads to metaphases in which chromosomes are not precisely orientated and to c-mitoses. Some of these metaphases lose any detectable MT assembly, others show altered spindles consisting of arrowhead-like structures, which have been interpreted as remnants of kinetochore MTs, or of small round structures at the kinetochore position, with or without connecting strands (Utrilla et al., 1989). Taxol on cultured plant cells, instead induces the formation of central aster-like arrays of MTs, multipolar spindles and multipartite phragmoplasts (Weerdenburg et al., 1986), which have not been observed in the present work. Hence, in our study, due to the resemblance with the colchicine-induced alterations, it is possible that 25 µM As directly caused conformational changes of the tubulin, leading to disassembly of the MTs, as colchicine does (Bhattacharyya et al., 2008).

Although it is known that As impairs spindle function causing chromosome condensation and c-mitosis (Panda and Panda, 2002), there is a lack of recent literature on this subject. Some available data about the effect of As on MTs come from animal/human models; however, the mechanism involved in the mitotic arrest is controversial. Even if most explanations focus on tubulin as the direct target of As, it is unclear if As promotes tubulin stabilization or inhibits tubulin polymerization. Besides, some authors hypothesize mechanisms independent of tubulin organization/polymerization (Taylor et al., 2008). Even though indirect effects of As are probably involved in the spindle functions, the direct effect of As on MTs has been demonstrated through experiments performed using a cell-free tubulin assembly assay. The results of these experiments have shown that only As (III) causes a significant inhibition of tubulin polymerization, suggesting that the effects of As (V) on mitosis are due to a cellular reduction of pentavalent to trivalent forms (Kligerman and Tennant, 2007).

Because of the rapid transformation of arsenate to arsenite in plant cells (Meharg and Hartley-Whitaker, 2002), it is likely that, in our experimental system, the MT alterations can be largely ascribed to arsenite. Experiments conducted in our laboratory growing pea seedlings with 25 µM of arsenite for 24 h, have confirmed this hypothesis. These experiments have, in fact, shown a remarkable increase in c-mitosis, and the alterations in the MT mitotic arrays were mostly similar to those induced by arsenate (seesupplementary data). However, some As (V) exists within plant root cells (Xu et al., 2007) and arsenate toxicity (e.g. through interference with phosphate) cannot be excluded with certainty.

Many metals impair spindle functions (Panda and Panda, 2002). High concentrations and/or a long exposure time often lead to a severe fragmentation of MTs (Eun et al., 2000, Dovgalyuk et al., 2003, Liu et al., 2009 and Xu et al., 2009). On the contrary, lower doses damage MT arrays with different outcomes in relation to the metal applied. Cadmium, in pea root apices, induces the formation of aberrant spindles that consist of coarse, irregularly thickened bundles of MTs. They rarely degenerate, as instead happens with As, and c-mitoses are scarce (Fusconi et al., 2007). Lead, at 20 µM, splits maize spindle poles into several minipoles (Eun et al., 2000). Aluminium delays MT disassembly during mitosis, inhibits spindle formation and induces atypical MT arrays (Frantzios et al., 2000), possibly due to a stabilizing action of aluminium on MTs (Dovgalyuk et al., 2003). These data point to specific interactions of the above mentioned elements with the cell cycle machinery, and not only to the affinity to the thiol groups of proteins.

In conclusion, according to our results and literature data, As may be considered as a mitotic poison which, like most metallic salts, affects the mitotic arrays of MTs. Its clastogenic effect is relatively low; in fact, DNA degradation increased only under high As concentrations, and chromosome aberrations, such as bridges in ana/telophase and fragmentations, were virtually absent. Understanding how these chemicals modify cell structures, may help scientists evaluate the results of short-term plant genotoxicity assays, in order to screen and monitor environmental pollution.

Acknowledgments

This paper was supported by the Centre of Excellence for Biosensing through the use of Plants and Microorganisms (CEBIOVEM) and by the Italian MURST.

Appendix A. Supplementary data

(A) Effects of 0.25, 2.5, 25 and 250 μM sodium arsenite on the root length of *Pisum sativum* seedlings, after 24 h of exposure. The pattern of growth is similar to that of roots treated for 24 h with the same concentrations of arsenate, but arsenite induced a stronger inhibition of growth from the concentration 2.5 μM. Differences between root lengths after administration of arsenate (text, Fig. 1A) and arsenite were not significant at the same concentrations, except for the treatment with the highest arsenite concentration (250 μM) that blocked almost completely root growth. (B–E″) Representative images showing the effects of 24 h of exposure to 25 μM arsenite on the preprophasic band (PPB) and the mitotic spindle of the apical cells of *Pisum sativum* roots, obtained with the methods described in the text. (A–E) Immunofluorescent labelled microtubules, (A′–E′) corresponding DAPI stained nuclei, (C″–D″) superimposed images of immunofluorescent labelled microtubules and DAPI stained nuclei. (A–B′) Fragmented and irregularly bundled PPB in an isodiametric (A–A′) and an elongated (B, B′) prophase cell. (C–C″) Irregular spindle, formed of thick bundles of MTs and (D–D″) c-mitosis with remnants of microtubules. Bar = 10 μm.

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