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Lead-induced DNA damage in *Vicia faba* root cells: Potential involvement of oxidative stress

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ABSTRACT

Genotoxic effects of lead (0–20 μM) were investigated in whole-plant roots of *Vicia faba* L., grown hydroponically under controlled conditions. Lead-induced DNA damage in *V. faba* roots was evaluated by use of the comet assay, which allowed the detection of DNA strand-breakage and with the *V. faba* micronucleus test, which revealed chromosome aberrations. The results clearly indicate that lead induced DNA fragmentation in a dose-dependant manner with a maximum effect at 10 μM. In addition, at this concentration, DNA damage time-dependently increased until 12 h. Then, a decrease in DNA damages was recorded. The significant induction of micronucleus formation also reinforced the genotoxic character of this metal. Direct interaction of lead with DNA was also evaluated with the a-cellular comet assay. The data showed that DNA breakages were not associated with a direct effect of lead on DNA. In order to investigate the relationship between lead genotoxicity and oxidative stress, *V. faba* were exposed to lead in the presence or absence of the antioxidant Vitamin E, or the NADPH-oxidase inhibitor dephenylene iodonium (DPI). The total inhibition of the genotoxic effects of lead (DNA breakage and micronucleus formation) by these compounds reveals the major role of reactive oxygen species (ROS) in the genotoxicity of lead. These results highlight, for the first time in vivo and in whole-plant roots, the relationship between ROS, DNA strand-breaks and chromosome aberrations induced by lead.

1. Introduction

Lead (Pb) is one of the most useful metals, and it is also one of the most toxic [1]. The widespread use of lead has caused global contamination of air, water and soil. Extensive research has found that lead can cause neurological, haematological, gastrointestinal, reproductive, circulatory, and immunological pathologies [2]. Moreover, the International Agency for Research on Cancer has listed inorganic lead compounds as possibly carcinogenic (Group-2B) chemicals [3,4]. In plants, lead has been described to influence various morphological, physiological and biochemical processes. Pb is known to strongly inhibit plant growth, root elongation, seed germination, seedling development, transpiration, chlorophyll production, lamellar organisation in chloroplasts and cell division [5,6]. However, only limited data are available on the effects of Pb on plant genetic material. It has been observed that Pb promoted single strand-breaks in lupine roots [7]. In a recent study, Gichner et al. [8] demonstrated that large concentrations of lead induced DNA strand-breaks. In *Vicia faba* roots, lead shortened the mitotic stage and prolonged the interphase, thus prolonging the cell cycle [6]. At low concentrations (below 10 μM) lead increased the mitotic index, but decreased it at higher concentrations. Pb also dose-dependently increased the micronucleus frequency [6,9].

Lead genotoxicity mechanisms are complex and not yet well understood. In vitro studies demonstrated that lead interacts with protein and nucleic acids, particularly at sulfhydryl groups and the phosphate backbone [10,11]. Lead decreases the fidelity of DNA polymerases and disturbs DNA and RNA synthases. Pb may also act as co-carcinogen by synergistically potentiating the genotoxicity of UV light and alkylating carcinogens [12]. In addition, ascorbic acid can reduce sister-chromatid exchange caused by lead, implying that free radicals may play a role in genotoxicity of this metal [13]. Using a plasmid-relaxation assay, Yang et al. [14] demonstrated that high concentrations of lead promote DNA strand-breakage and the formation of 8-hydroxydeoxyguanosine (8-OHdG) adducts in DNA.

In previous work, we demonstrated that an early step in the lead-induced oxidative burst in *V. faba* roots is strongly associated with activation of NADPH-oxidase and production of $O_2^{\bullet -}$ [15]. It is assumed that lead induces DNA damage in mammalian

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cells through excessive production of ROS and aldehydes, such as malondialdehyde (MDA) [16]. However, in plants, DNA damage-induction mediated by lead remains unclear. The aim of this work was to investigate the role of ROS in lead-induced genotoxicity. For this purpose, DNA damage was studied in lead-treated plants, in the presence or absence of Vitamin E, a powerful scavenger of ROS and a soluble lipid by-product of oxidative stress [17] and DPI, an NADPH oxidase inhibitor [15]. Pb-induced DNA alterations in *V. faba* root cells were evaluated by use of (i) the comet assay, which allowed the detection of DNA strand-breaks and (ii) the *V. faba* micronucleus test (MN), which revealed chromosome aberrations. Direct interactions between lead and DNA were also evaluated with the a-cellular comet assay.

2. Materials and methods

2.1. Materials

The *V. faba* L. cultivar "aguadulce" (Tezier, France) was used for this study. Dry seeds were soaked for 24 h in de-ionized water. The seed coats were removed and placed on moistened filter paper in a germination chamber under optimal conditions of germination, i.e. in the dark at 22 ± 1 °C and 100% humidity. After 3 days, when the primary roots were about 2–3 cm in length, the seedlings were transferred to a PVC tank (3 plants per tank) containing continuously aerated Hoagland nutrient solution. Four days were necessary to obtain secondary roots of suitable length (1–2 cm) for the different tests. For experiments, roots were exposed to different concentrations of Pb(II) ($Pb(NO_3)_2$; Sigma-Aldrich chemical), in the presence or absence of Vitamin E or DPI (Sigma-Aldrich chemical). All plants were grown and exposed under controlled conditions during a 16-h photoperiod at 70% relative humidity and day/night temperatures of 24/22 ± 1 °C. Light was supplied by 600-W Osram Nav-T Super High Pressure Sodium Lamps providing a minimum photosynthetic photon-flux density of 500 $\mu mol\ m^{-2}\ s^{-1}$ at the top of the plant [15].

2.2. Cellular comet assay

Single-cell gel electrophoresis (SCGE) was performed according to Gichner et al. [18] with some modifications. For dose–response experiments, *V. faba* roots were treated at 20 °C in the dark, with different $Pb(NO_3)_2$ concentrations (1, 5, 10, and 20 μM) for 8 h. For kinetic experiments, roots were exposed to 10 μM $Pb(NO_3)_2$ for 1, 4, 8, 12, 16 and 20 h. To evaluate the implication of ROS in lead-induced genotoxicity, some plants were co-incubated with Vitamin E (1 and 10 μM) or DPI (0.1 and 1 μM), an inhibitor of the oxidative burst induced by lead in *V. faba* root tips [15]. In order to compare results obtained with those from the normalized micronucleus test, plants were incubated with lead (10 μM) for 6 h. Some plants were also pretreated with Vitamin E (1 or 10 μM) or DPI (0.1 or 1 μM) for 1 h before exposure to lead, while others were only treated during 7 h with Vitamin E or DPI at the same concentrations as previously described. In each experiment, negative controls were placed in Hoagland's solution without lead for the same exposure times, while positive controls were incubated with 35 μM H_2O_2 for 1 h. It is noted that maleic hydrazine (MH) cannot be used as a positive control in the comet assay [19]. After treatment of the seedlings, excised roots were rinsed in water and placed in a 60-mm petri dish kept on ice and spread with 1.5 mL of cold PBS buffer (NaCl 130 mM, Na_2HPO_4 7 mM, NaH_2PO_4 3 mM, EDTA 50 mM, pH 7). The roots were gently sliced with a fresh razor blade. The plate was kept tilted in the ice so that the isolated root nuclei would collect in the buffer. The suspension with released nuclei was cleaned of debris by filtering through a 20-μm nylon cloth. All operations were conducted under inactinic red light to avoid light-induced damage.

Then comet-assay slides were prepared according to Gichner et al. [18]. For each slide, 50 randomly chosen nuclei were analyzed with an Olympus fluorescence microscope with an excitation filter of 510–560 nm and a barrier filter of 590 nm. A computerized image-analysis system (Komet version 5.1, Andor Technology) was employed. The percentage DNA in tail was used as the primary measure of DNA damage according to Hartmann et al. [20]. Three slides were evaluated per treatment and each treatment was repeated at least three times. From the repeated experiments, the averaged median value for the percent DNA in tail was calculated for each treatment group from the DNA per cent value of each slide [21].

2.3. A-cellular comet assay

SCGE slides with nuclei from untreated root cells were prepared as outlined above and the slides were immersed in solutions of 1 M NaCl, 10 mM Trizma containing 1, 5, 10 or 20 μM $Pb(NO_3)_2$ for 1 h at 4 °C. Negative control slides were immersed in the same solution without lead, while positive controls were incubated in the same solution with 8 μM H_2O_2. After the treatment period, the slides were rinsed three times for 5 min by immersion in cold 400 mM Tris buffer, then electrophoresed and analyzed as described earlier.

2.4. Micronucleus test

The *V. faba* MN test was carried out according to Ma et al. [22]. Exposure time was 30 h for the negative control, and 6 h for $Pb(NO_3)_2$-treated groups, followed by a 24-h recovery period. The test concentrations for $Pb(NO_3)_2$ were 1, 5, 10 and 20 μM. To evaluate the potential role of oxidative burst in lead-induced MN formation, *V. faba* roots were pre-treated with 10 μM Vitamin E or 1 μM DPI, 1 h before treatment with lead. The direct effects of these two substances were evaluated by exposure of *V. faba* roots to Vitamin E or DPI during 7 h, followed by a 24-h recovery period. For each experiment, five seeds were used per treatment. Maleic hydrazine (MH; 40 μM) was used as a positive control. Aerated Hoagland's solution was used as a negative control. After treatment, root tips were fixed in Carnoy's solution (glacial acetic acid/ethanol 1:3) at 4 °C for one night, and transferred to 70% ethanol for storage. Root tips were then hydrolyzed in 1 N HCl at 60 °C for 5–7 min. Five slides were prepared for each of the five seeds. After staining the root tips with 1% aceto-orcein, the interphase cells were scored for micronucleus frequency under a 1000× magnification. Five thousand cells per tip were counted. In order to avoid under-estimation of micronuclei due to impaired cell-proliferation rate [22], the MN test was performed only on root tips with a mitotic index superior to 2%.

2.5. Analysis of lead content

Lead content was analyzed as previously described [15]. *V. faba* plants roots were exposed to 10 μM of lead nitrate in Hoagland solution during different time periods (0, 4, 8, 12, 16 and 20 h). Roots were collected and rapidly washed in distilled water. Lead bound to the rhizoderm was removed as follows: fragments were shaken with 40 mL of 0.001 M HCl during 3 min, and 360 mL of 1 M HCl were added to yield a final concentration of 0.01 M HCl. After shaking for another 5 min, roots were washed in distilled water, oven-dried at 80 °C for 48 h, and then weighed. Roots were mineralized in a 1:1 mixture of 65% HNO_3 and 30% H_2O_2 at 80 °C during 6 h. After filtration, lead concentrations were determined with an IRIS Intrepid II XDL ICP-OES. The accuracy of the analytical procedure was verified with a reference material: Virginia tobacco leaves (CTA-VTL-2, polish certified reference material; ICHTJ).

2.6. Statistical analysis

For each experiment, statistical analysis was performed with a one-way ANOVA and the multiple comparison method of Tukey [23].

3. Results

3.1. DNA-damage analysis by use of the cellular comet assay

Fig. 1 illustrates the DNA-damaging effect of lead applied for 8 h on *V. faba* roots. The positive control (PC) was obtained by incubation of roots with 35 μM of H_2O_2. Under these conditions, H_2O_2 induced 76 ± 2.5% DNA in tail. Cellular comet-assay data clearly demonstrate a significant increase in DNA fragmentation correlated with increasing concentrations of lead (1–20 μM). The percentage DNA in tail increased from 23 ± 1.2% (negative control) to 64 ± 2.1% for 20 μM lead treatment ($P < 0.01$). Under these experimental conditions, the maximum effect was observed after exposure to 10 μM of lead.

3.2. Evaluation of chromosome aberrations

Chromosome aberrations analyzed with the micronucleus (MN) test are shown in Fig. 2. It is worth noting that 20 μM of lead induced a blackening of the root tips and a loss of mitosis. Under these conditions, micronuclei were not quantified. Results in Fig. 2A illustrate that 1, 5 and 10 μM $Pb(NO_3)_2$ had no significant effect on cell mitosis. Data shown in Fig. 2B demonstrate that lead dose-dependently induced MN formation in *V. faba* root tips. The MN induction became significant with 5 μM of lead (7.2 ± 0.15‰). The maximum effect was observed with 10 μM (8.2 ± 0.3‰). This MN value was slightly higher than the positive control obtained with MH (6.45 ± 0.21‰).

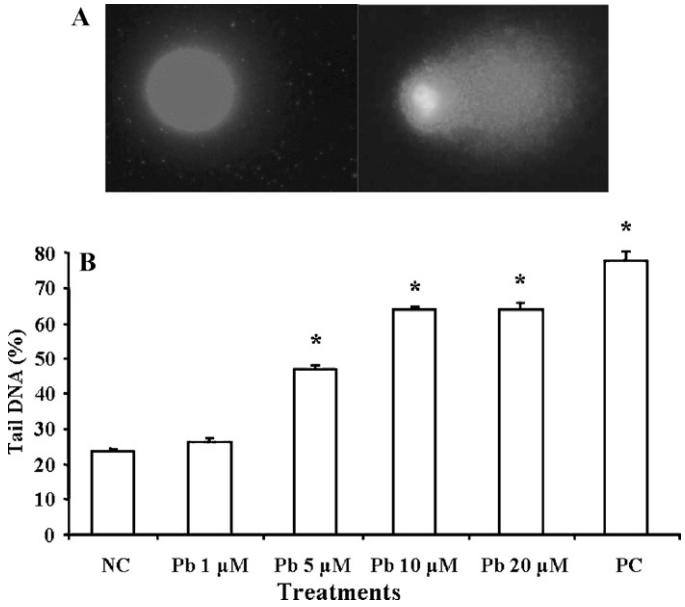

Fig. 1. (A) Effects of lead on *V. faba* root-cell DNA: undamaged (left) and damaged (right) nuclei (×400). (B) Dose-effect of lead on DNA strand-breakage assessed with the comet assay in whole *V. faba* roots. The negative control (NC) was realized by incubation of roots in Hoagland's solution without lead for the same exposure duration (8 h). The positive control (PC) was realized by incubation of roots with 35 µM H_2O_2 in Hoagland's solution during 1 h. Each treatment was repeated five times. Each value represents the mean ± SE of three separate experiments. *Values significantly different from the control ($P < 0.01$).

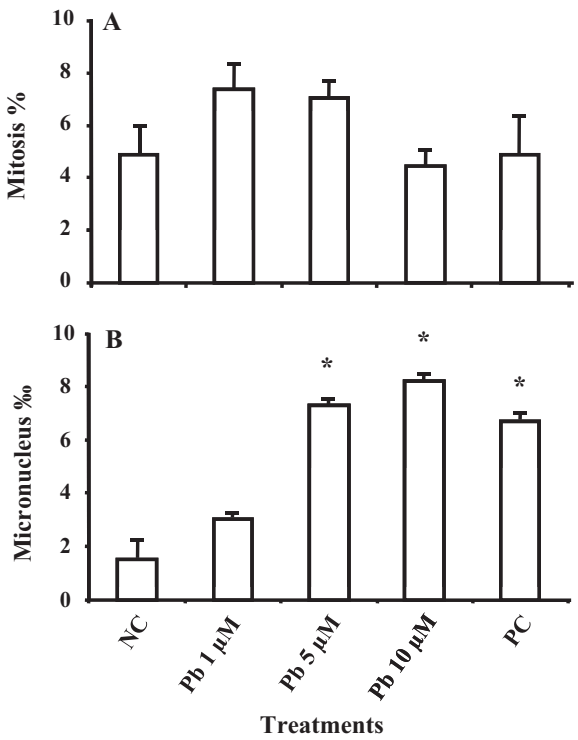

Fig. 2. Mitotic index (A) and micronucleus frequency (B) values in *V. faba* roots exposed to different concentrations of lead (1, 5, and 10 µM). NC: negative control, PC: positive control. Each value represents the mean ± SE of five seeds in three separate experiments. *Values significantly different from the control ($P < 0.01$).

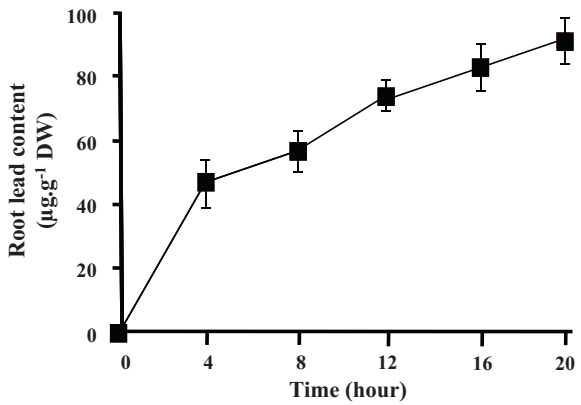

Fig. 3. Kinetics of lead uptake into roots exposed to 10 µM of lead nitrate in Hoagland's solution. Lead concentrations in the roots were evaluated by means of ICP-OES. Each point represents the means of lead concentration ± SE of five experiments.

3.3. Kinetics of lead uptake and lead-induced DNA damage in *V. faba* roots

To investigate the relationship between DNA fragmentation and Pb concentration in the roots, the kinetics of lead accumulation (10 µM) were analyzed (Fig. 3). The concentration of 10 µM lead was chosen because it induced the maximum genotoxic effect on *V. faba* roots without blackening of root tips. Data reveal that the entry of lead into the roots was very fast between 0 and 4 h (11.25 $\mu g\,g^{-1}$ dry weight h^{-1}), and reached 46.5 ± 6.5 $\mu g\,g^{-1}$ dry weight. Then, lead uptake decreased to 2.53 $\mu g\,g^{-1}$ dry weight h^{-1} and became constant. Under these experimental conditions the maximum lead content was reached at 20 h (90.2 ± 6.9 $\mu g\,g^{-1}$ dry weight).

As exposure time is fixed in the normalized MN test, it cannot be used for the kinetic exposure experiment. So, the comet assay was used to evaluate the kinetics of DNA-damage induction by 10 µM lead nitrate (Fig. 4). Results show that, after 4 h of exposure, DNA strand-breaks increased of about 14% in comparison with the control. After 4 h of incubation with lead, a strong and significant increase in DNA strand-breaks was recorded, with a maximum

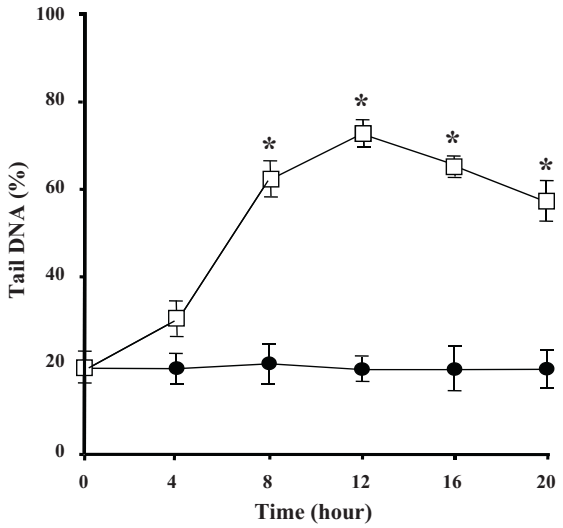

Fig. 4. Time-dependent DNA strand-breakage induced by lead nitrate in whole plant roots. *V. faba* roots were incubated with (□) or without (●) 10 µM lead nitrate in Hoagland's solution. Each value represents the mean ± SE of five plants in three separate experiments. *Values significantly different from the control ($P < 0.01$).

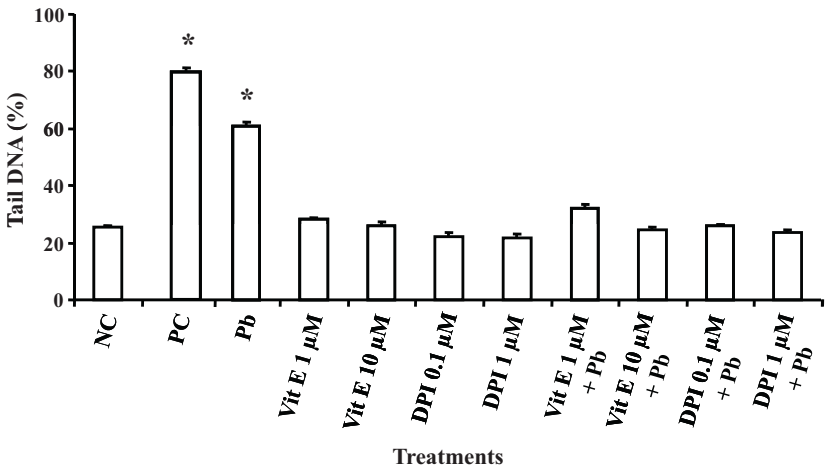

Fig. 5. Effect of the ROS scavenger Vitamin E or the NADPH-oxidase inhibitor DPI on lead-induced DNA strand-breakage in *V. faba* whole plant roots. *V. faba* plant roots were incubated with Vitamin E (1 and 10 μM) or DPI (0.1 and 1 μM) in culture medium 1 h before addition of lead nitrate (10 μM). NC: negative control, PC: positive control. Each value represents the mean ± SE of five plants in three separate experiments. *Values significantly different from lead treated roots ($P < 0.01$).

effect at 12 h (72 ± 1.7% DNA in tail). After this time, DNA strand damage decreased until 20 h (58.3 ± 3.5%).

3.4. Analysis of DNA damage with the a-cellular comet assay

In order to evaluate the potential direct effects of lead on *V. faba* root-cell DNA, the a-cellular comet assay was performed for 1 h. Treatment longer than 2 h cannot be applied in the a-cellular comet assay as even control nuclei become severely damaged, resulting in a strong increase in the percentage DNA in the tail [18]. Results obtained with the a-cellular comet assay reveal that 8 μM H_2O_2 induced 83 ± 3.1% DNA in tail after 1 h of incubation (data not shown). Under the same experimental conditions, whatever the concentration, lead did not directly induce DNA damage (28 ± 1.7% for the control vs. 30.2 ± 1.5% for 20 μM lead).

3.5. Effects of Vitamin E and DPI on lead-induced DNA fragmentation and micronucleus formation

To identify the potential role of ROS in lead-induced genotoxic effects, *V. faba* roots were pre-incubated with vitamin E or DPI during 1 h before exposure to lead. The results (Fig. 5) indicate that lead-induced DNA fragmentation (59.3 ± 1.8% in tail) was dose-dependently inhibited by vitamin E: with 1 μM, a reduction of 90%in DNA fragmentation was observed, while 10 μM Vitamin E totally abolished it. Similarly, at the two test concentrations used, DPI totally inhibited lead-induced DNA breakdown. Under these conditions, the percentages of DNA in tail were similar to those in the negative control. Treatments of roots with Vitamin E or DPI without exposure to lead did not influence root-cell DNA fragmentation.

The effects of vitamin E and DPI were also evaluated in the MN test (Fig. 6). Results show that 10 μM vitamin E alone significantly decreased the MN frequency (0.5 ± 0.1‰) in comparison with the control (1.95 ± 0.2‰), without modification of the cell mitosis. Vitamin E completely inhibited the effect of lead (10 μM) on MN induction (0.9 ± 0.2‰). Under the same conditions, DPI (1 μM) alone had no effect on the basal level of MN, while it significantly inhibited the effects of lead on MN induction (1 ± 0.1‰). These data clearly illustrate the potential role of oxidative stress in lead-induced MN formation.

4. Discussion

Even though lead toxicity in plants is well known [24], only few studies have examined its genotoxic effects. Relevant in vivo studies on DNA damage by lead in plant systems are lacking. Gichner et al. [8] showed that lead could induce somatic mutations and DNA strand-breaks in tobacco plants. In a recent study we observed that the genotoxicity of lead was associated with its chemical speciation [9]. In the present work, data of the cellular comet assay illustrate the DNA-damaging effect of lead (Fig. 1). These results indicate that the DNA damage observed in *V. faba* roots are correlated with the external lead concentration, as previously described with high lead concentrations [7,8]. The maximum effect was observed with 10 μM of lead. The alkaline comet assay reliably detects single-strand (SS) and double-strand (DS) DNA breaks, even at very low damage levels. SS and DS DNA breaks result from numerous reaction types: base- and nucleotide-excision repair, direct scission of the DNA backbone by chemical or radical attack, scission following the binding of an intercalating agent, the action of endonuclease or topoisomerase, the presence of alkali-labile DNA adducts or alkali-labile sites [25].

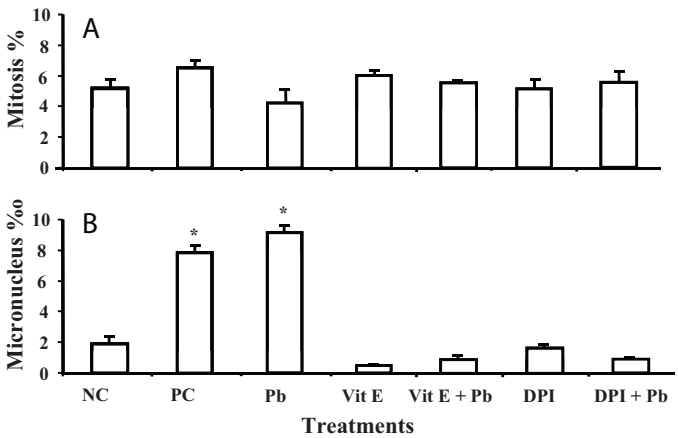

Fig. 6. Mitotic index (A) and micronucleus frequency (B) values in *V. faba* roots exposed to lead nitrate (10 μM) in the presence or absence of Vitamin E (10 μM) or DPI (1 μM). NC: negative control, PC: positive control. Each value represents the mean ± SE of five seeds in three separate experiments. *Values significantly different from the control ($P < 0.01$).

Chromosome aberrations analyzed by the MN test indicate that lead dose-dependently induced MN formation in *V. faba* root tips (Fig. 2B). The maximum effect was obtained at 10 μM (8.2 ± 0.3‰) as previously observed using the cellular comet assay. These data also illustrate the genotoxic potential of lead [26]. The *V. faba* MN test is a very sensitive and useful method that allows detection of both clastogenic and aneugenic effects [27,28]. Micronuclei are the result of chromosome breaks (or mitotic anomalies) that require a passage through mitosis to be recognisable. The molecular mechanism of MN induction is not yet clearly understood. Under our experimental conditions, the same kind of dose-dependent response observed in the two tests (comet assay and MN test) suggests a close link between lead-induced DNA damage and MN formation.

To determine the potential relationship between lead concentration in roots and genotoxicity, lead (10 μM) uptake into the roots was analyzed as a function of time (Fig. 3). Our data illustrate the fast uptake and accumulation of lead into *V. faba* roots during the first hours of treatment. The kinetics of lead accumulation in *V. faba* roots are in agreement with those found by Nedelkoska and Doran [29] in several plants treated with Cd, Ni or Cu. Lead accumulation in plant roots has been demonstrated by many authors [6]. It has been reported that lead effects are directly related to exposure dose and duration [7,26]. However, while lead was still taken up after 12 h of culture, a decrease in DNA damage was observed (Figs. 3 and 4). If these results seem directly correlated to induction of DNA-repair systems, this could also be associated with a rapid and efficient system of lead detoxification in plants [30]. This system includes chelation by organic or amino acids [31] or by phytochelatins [32] and sequestration in vacuole [33] or plasma-tubule [30]. Although it has been observed that Pb detoxification was quickly activated [30,32], our results indicate that these detoxification and repair systems became efficient only after 12 h of exposure to lead.

In order to determine pathways involved in lead genotoxicity, the direct effect of lead on DNA was assessed with the a-cellular comet assay. Results reveal that lead did not directly induce DNA damage after 1 h of incubation (data not shown). These results confirm those of Valverde et al. [34], who did not find DNA damage due to direct DNA–lead interaction in different mouse-cell types. These data are also in agreement with those of Hartwig [35], who proposed that direct genotoxic effects of metals are rather weak and/or restricted to high concentrations (more than 1 mM). Our results suggest that lead genotoxicity was mediated by another indirect pathway. In view of the lead-induced ROS production in plant roots [15,36] and according to Rucinska et al. [7], the induction of an indirect mechanism, such as oxidative stress is suggested. It appears likely that many of the genotoxic effects of lead in mammalian cells are mediated by ROS and/or lipid-soluble by-products of oxidative stress such as MDA.

To identify the potential role of ROS in the induction of DNA alterations, *V. faba* plant roots were incubated with two concentrations of Vitamin E or DPI, during 1 h before exposure to lead. Fig. 5 results suggest the implication of ROS in lead-induced DNA strand-breaks. Vitamin E and the NADPH oxidase-inhibitor DPI completely abolished the increase in the percentage DNA in tail after lead treatment. At the same time, neither Vitamin E nor DPI influenced lead accumulation kinetics in *V. faba* plant roots (data not shown). Vitamin E is a fat-soluble vitamin that protects cells against ROS [37]. Recently, Collin et al. [38] demonstrated that Vitamin E is essential for the tolerance of *Arabidopsis thaliana* to metal-induced oxidative stress. In a previous work, we highlighted the fundamental role of NADPH oxidase in lead-induced ROS production in *V. faba* root tips [15]. In these plant roots, ROS production was totally inhibited by 0.1 or 1 μM DPI, a well-known inhibitor of NADPH oxidase. In the present work, data obtained with DPI and Vitamin E illustrate a relationship between oxidative stress and lead-induced DNA strand-breakage. In *in vitro* system, it has been demonstrated that singlet oxygen is the major species participating in the induction of DNA strand-breakage and 8-hydroxydeoxyguanosine adduct induction by lead [14]. In addition, the •OH radical is considered to be the ultimate reactive oxygen species that interacts with DNA and promotes genetic damage [39]. This radical attacks DNA on the sugar residue and induces DNA fragmentation, base loss, and strand-breaks with a terminal sugar residue fragment [40]. It also produces thymine glycol, 7,8-dihydro-8-oxoguanine or 2,6-diamino-4-hydroxy-5-formamido-pyrimidine.

Because lead can promote oxidative stress, DNA strand-breaks and chromosome aberrations, the MN test was also conducted in the presence of Vitamin E or DPI. Fig. 6 shows that these compounds completely inhibited the effect of lead on MN formation. These data strongly support the role of oxidative stress in lead-induced MN formation in *V. faba* root tips. According to Figs. 5 and 6, genotoxic effects of lead were rapid to appear in *V. faba* root tips. Six hours of exposure were sufficient to increase both DNA fragmentation (48% DNA in tail) and micronucleus formation. These results underline the role of DNA fragmentation in MN formation induced by lead. Overall, these data highlight for the first time in a plant a possible relationship between lead, oxidative stress, DNA fragmentation and micronucleus formation. However, genotoxic processes are very complex, and epigenetic effects could also be involved in lead-induced MN formation [41]. Indeed, lead is able to interfere with the spindle apparatus of dividing cells and promotes genotoxic effects. In this work, we cannot totally exclude this hypothesis.

In conclusion, these data strongly suggest that, in whole plant roots, lead genotoxicity could be mediated by ROS production. The use of Vitamin E and DPI helps to establish a link between ROS production, DNA fragmentation and probably micronucleus formation. Although metallic salts are effective mitotic poisons due to their affinity for thiol groups, the total inhibition of lead-induced MN formation in the presence of Vitamin E or DPI strongly suggests the implication of ROS in MN induction. If our results underline the role of oxidative stress in DNA strand-breakage, we cannot rule out that lead genotoxicity and especially MN formation are almost exclusively mediated by ROS. In this work, it was also impossible to discern the precise nature of the radical that directly or indirectly interacts with DNA. Lipid-oxidation products quenched by Vitamin E could be also associated with DNA-adduct formation and breakages. Other research is still necessary to fully understand the complete genotoxic mechanism.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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