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Full Length Article

Effects of Exogenous Application of Ascorbic Acid on Genotoxicity of Pb in *Vicia faba* Roots

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Abstract

Effects of ascorbic acid (AsA) were investigated on *Vicia faba* roots exposed to different concentration of Pb $(NO_3)_2$. Under all the Pb concentrations (10, 20, 40 μM) treatment, micronucleus (MN) frequency was higher when compared to the control. Furthermore, chromosomal aberration (CA) frequency increased, but not of mitotic index ($P < 0.05$). The application of the AsA (0, 0.5, 1, 2, 4 mM) decreased markedly MN frequency and CA frequency, and increased mitotic indexes under Pb treatments. The results indicated that exogenous application of AsA exerted a positive role on *V. faba* roots exposed to Pb stress. © 2014 Friends Science Publishers

Keywords: *Vicia faba*; Chromosomal aberratins; Mitotic activity; Ascorbic acid; Pb stress·

Introduction

Lead (Pb) is a toxic heavy metal which can be taken up by plant roots from soil and impact on human health through food chain (Shotyk and Le Roux, 2005). Pb has no biological function and induces a broad range of deterioration effects on plant morphological, physiological and biochemical processes due to strongly phytotoxic during plant growth and development. For example, excess Pb causes plant growth retardation, inhibits root elongation, seed germination, photosynthesis, and damages membrane structure (Islam *et al*., 2008; Li *et al*., 2012). Moreover, increasing evidence indicates that Pb toxicity is attributed to its oxidative damage (Pourrut *et al*., 2011). Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidases (APX) were employed to mitigate and repair the ROS damages (Edreva, 2005). Antioxidants such as reduced glutathione, vitamin E and ascorbic acid (AsA) are also employed to counter ROS (Das *et al*., 2012).

Ascorbic acid (AsA) is known to be a low molecular weight and an important water-soluble antioxidant molecule for enzymatic detoxification. Ascorbic acid is also as an ascorbate peroxidase substrate to scavenge H_2O_2 in the chloroplast stroma (Gadallah, 2000; Shigeoka *et al*., 2002) and protects or regenerates oxidized carotenes and α-tocopherols (Noctor and Foyer, 1998). Ascorbic acid plays a regulatory role in the plant growth by modulating the synthesis of plant hormones such as ethylene, GA and ABA (Nambara and Marion-Poll, 2003; Pastori *et al*., 2003), and also plays important roles under abiotic stress, i.e., exogenous application of AsA enhanced plant tolerance to salinity stress, heavy metal Cd, chill and drought stress (Shalata and Neumann, 2001; Guo *et al*., 2005; Sajid and Aftab, 2009; Chao and Kao, 2010).

Although many studies have been conducted on roles of exogenous application of AsA under abiotic stress conditions, the function of exogenously applied AsA on *Vicia faba* root tip cells exposed to Pb stress has not been evaluated. It is reported that Pb interfered with the mitosis process and inhibited cell division of plant root tip cells (Shahid *et al*., 2011). Accordingly, in this study we investigated effects of AsA on *V. faba* roots exposed to Pb stress.

Materials and Methods

Materials and Treatments

Some *V. faba* seeds were surface sterilized in 0.5% (v/v) sodium hypochlorite and washed with distilled water for 3 times. Seeds were then soaked in different concentrations Pb $(NO_3)_2$ solutions (0, 5, 10, 20, 40 μM) for 24 h and transferred to Petri dishes for germination. Germinated seeds were cultivated hydroponically on Hoagland's nutrient solution consisting of 1 mM $MgSO_4$, 5 mM Ca $(NO_3)_2$, 0.2 mM KH_2PO_4, 50 μM H_3BO_3, 0.1 mM $(NH_4)_6Mo_7O_{24}$, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 10 μM Fe-EDTA, 5 mM KNO_3 and 0.3 μM $CuSO_4$ at pH 6.The nutrient solution was renewed every 3 days. Root length was measured after culturing 7 days.

Some seeds were soaked in the AsA (0, 0.5, 1, 2, 4 mM) solutions for 24 h, then transferred to Petri dishes and

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incubated. Distilled water was renewed once at 12 h intervals. Growing about 1 cm, the roots were cultured in different $Pb(NO_3)_2$ concentrations (0, 10, 20, 40 μM) for 6 h respectively. The other seeds were soaked in different $Pb(NO_3)_2$ concentrations (0, 10, 20, 40 μM) for 6 h, 12 h and 24 h, respectively. After $Pb(NO_3)_2$ solutions treatment, roots were washed with distilled water, reculturing for 24 h.

Slide Preparation, Staining and Scoring

During cell division crest-time, the primary root tips were cut about 1 cm from recultured seeds, placed in Carnoy's fixation fluid for 24 h, and stored in 70% ethanol. The root tips were first washed in distilled water for 5 min and hydrolyzed with 1 M HCl for 13 min at 60ºC. After HCl treatment, the root tips were soaked in distilled water for 1 min. Finally, the root tips stained with Feulgen for 30 min in dark. The root cap was removed and meristematic tissues were squashed on slides. The MN, mitotic and CA were counted microscopically. The MN had a chromatin structure similar to the main nucleus and appeared within the cytoplasm. The MN diameter did not exceed 1/3 of the main nucleus and was on the same plane focus. Chromosomal aberration (CA) consisted of micronucleus, lagging chromosomes, chromosome fragment, chromosome bridge, etc.

Statistical Analysis

Ten tips (2000 cells in per tip) were utilized in each different treatment. The results were expressed as the values ± standard deviation (SD).All Data were analyzed with the statistical package SPSS11.0.

Results

Several different Pb^{2+} concentrations (0, 5, 10, 20 and 40 μM) were checked to research circumstance of Pb^{2+} damage on *V. faba* root length. The root length of 5 μM Pb^{2+} treatment was the longest in all treatments, which was much longer when compared to control (Fig. 1). However, other root length here in tested was pronouncedly shorter than control. The higher Pb^{2+} concentrations inhibited the growth of *V. faba* roots.

Based on the analysis of *V. faba* root growth, 10, 20 and 40 μM Pb^{2+} concentrations were used to investigate the relationship between genotoxicity and Pb concentration. Micronucleus (MN) analysis was performed to estimate genetic effects of chemicals during mitotic activity on *V. faba*. It was observed that MN frequency was significantly higher than control after treatment with distinct concentrations of Pb (Table 1), and also relatively increased with increasing Pb concentration. MN induction was significantly detected ($P< 0.01$) after Pb treatment 6 h and MN frequency relatively increased with prolongation of Pb treatment time. Application of AsA decreased markedly ($P<$ 0.05) MN frequency of *V. faba* root tip cells exposed to different Pb concentrations (Table 2). There were the lowest values on inhibiting MN frequency at 1 mM AsA. Namely, MN production decreased with AsA concentration changing from 0.5 mM to1 mM, but relatively increased from1 mM to 4 mM.

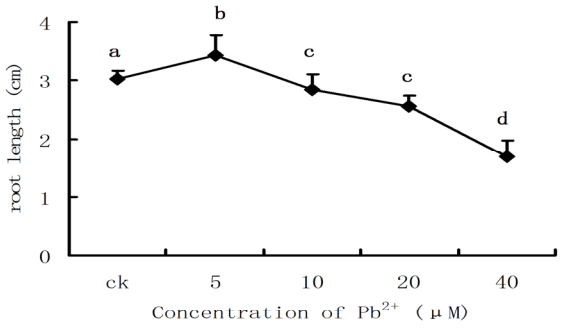

Fig. 1: Effect of Pb^{2+} on root length of *Vicia faba*

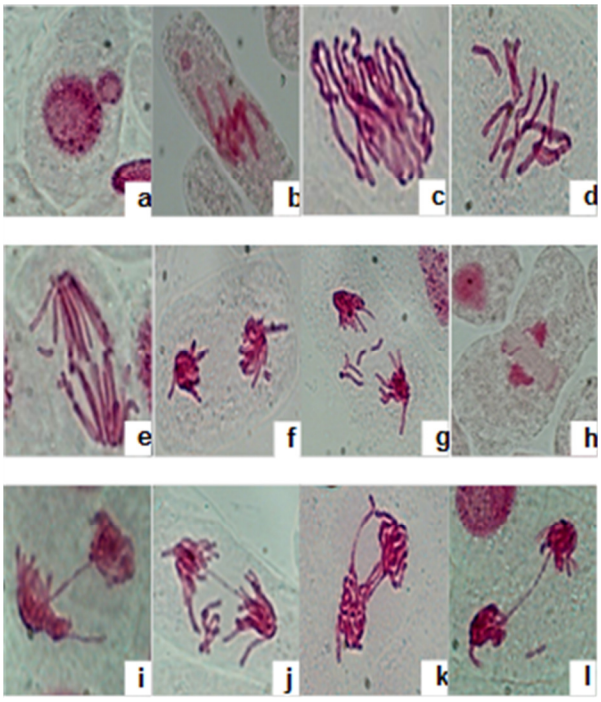

Fig. 2: Chromosomal aberrations of root tip cells of *V. faba* germinated in different Pb concentrations

a. Single micronucleus in interphase; **b**. Single micronucleus in metaphase; **c**. alignment metaphase; **d.** laggard Chromosome in metaphase; **e.** Chromosome fragment in anaphase; **f.** Chromosome lagging in anaphase; **g.** chromosome fragments in late anaphase; **h.** Chromosome fragment in telophase; **i.** Chromosome single bridge in anaphase; **j.** Chromosome lagging and single bridge in anaphase; **k.** Chromosome bridges in anaphase; **l.** Chromosome fragment and single bridge in anaphase.

The genotoxic activity of Pb stress also showed chromosomal aberration, which concomitantly occurred with MN. With increasing Pb concentration and

Table 1: Effects of different concentration Pb in *V. faba* root tip cells

Pb^{2+}	Micronucleus Frequency			Chromosome Aberration Frequency			Mitotic Index		
μM	x ± S (‰)			x ± S (%)			x ± S (%)		
	6h	12h	24h	6h	12h	24h	6h	12h	24h
0	1.03± 0.25	1.18± 0.14	0.96± 0.22	0.22± 0.08	0.19± 0.03	0.24± 0.15	6.31± 1.02	6.36± 2.27	6.79± 1.49
10	7.97± 2.13**	9.24± 1.96**	13.41± 3.07**	8.65± 1.59**	12.91± 2.32**	17.42± 2.23**	4.96± 1.84*	4.31± 2.18*	3.82± 0.85**
20	11.36± 1.98**	14.81± 3.21**	17.32± 2.11**	11.37± 2.21**	14.45± 2.53**	19.31± 1.58**	4.27± 1.47*	3.84± 1.24**	3.14± 1.02**
40	13.25± 1.53**	14.22± 2.75**	14.54± 2.63**	10.45± 1.84**	13.15± 1.74**	12.64± 3.53**	2.85± 0.79**	2.61± 0.66**	1.94± 0.76**

*, **Compared with control, $P < 0.05$, $P < 0.01$

Table 2: Effects of different concentration of AsA and Pb in *V. faba* root tip cells

AsA mM	Pb^{2+} μM	Micronucleus Frequency x ±S	Chromosome aberration frequency x ±S	Mitotic index x ±S
0	10	7.97 ± 2.13	8.65 ± 1.59	4.96 ± 1.84
0.5	10	5.43±1.01*	6.24 ± 1.37*	6.78 ± 2.32*
1	10	4.36±2.21**	3.96 ± 0.83**	8.27 ± 2.4**
2	10	4.69±1.37**	5.38±1.1**	7.93 ± 1.2**
4	10	4.88±2.26**	6.03 ± 2.03*	6.45 ± 2.13*
0	20	11.36 ± 1.98	11.37 ± 2.21	4.27 ± 1.47
0.5	20	8.54 ± 1.43*	8.79 ± 0.87*	5.69 ± 1.3*
1	20	6.42 ± 2.36**	6.01 ± 1.64**	7.46 ± 2.71**
2	20	6.97±1.66**	6.65 ± 2.2**	7.52 ± 1.84**
4	20	7.34 ± 2.03**	5.32 ± 1.73**	6.31 ± 1.47*
0	40	13.25 ± 1.53	10.45 ± 1.84	2.85 ± 0.79
0.5	40	11.25 ± 1.31*	8.03 ± 2.09*	3.25 ± 0.63*
1	40	9.71 ± 1.97**	7.72 ± 2.03**	4.02 ± 1.6**
2	40	9.53 ± 2.27**	7.26 ± 1.38**	3.76 ± 0.95**
4	40	11.65 ± 1.56*	9.54 ± 1.57	3.19 ± 2.1*

*, **Compared with non-AsA treatment, $P < 0.05$, $P < 0.01$

prolongation of Pb treatment time, CA frequency gradually increased (Table 1). Different type of chromosomal aberrations was induced in the whole cell cycle, such as the presence of micronucleus (Fig. 2a, b), alignment chromosomes (Fig. 2c), laggard chromosomes (Fig. 2d, f), chromosome fragment (Fig. 2e, g, h) and chromosome bridge (Fig. 2i, j, k, l), etc. A significant difference between application AsA and non-application AsA was observed in CA frequency (Table 2). The CA frequency gradually decreased when AsA concentration changed from 0.5 mM to 1 mM.1 mM AsA treatment had the lowest CA frequency under different Pb^{2+} concentration.

The mitotic indexes treated with different Pb concentrations significantly decreased ($P < 0.05$) in comparison with control (Table 1). Moreover, mitotic indexes decreased with increasing Pb concentration and with prolongation of Pb treatment time. The exogenous application of AsA had increased the mitotic indexes than that of Pb alone treatment (Table 2). The mitotic indexes of 1 mM AsA treatment was the highest under different Pb^{2+} concentration. However, when treatment concentration was 4 mM AsA, the mitotic indexes began to decrease.

Discussion

It is well known that Pb stress induces many deterioration effects on plant morphological, physiological and biochemical processes. However, little information is about effects of Pb stress on plant genotoxicity. In the present study, MN frequency and CA frequency relatively increased with increasing Pb concentration and prolongation of Pb treatment time. This indicated a direct correlation between Pb dose and MN and CA frequency, which was consistent with some studies (Rucinska *et al*., 2004; Shahid *et al*., 2011). Increasing Pb concentrations strengthened the cell toxicity and seriously disturbed normal metabolic activity. Different type of chromosomal aberrations induced in the whole cell cycle, such as the presence of micronucleus, lagging chromosomes, chromosome fragment, chromosome bridge, etc. The results indicated Pb stress directly affected DNA duplication, disturbed RNA transcription and protein synthesis involved in the cell cycle. Shahid *et al.* (2011) reported that Pb also broke single and double strands of DNA and affected horizontal DNA–DNA or DNA–protein links. However, several literatures reported that Pb genotoxicity was due to oxidative stress, which was

mediated by ROS and/or MDA (Rucinska *et al*., 2004; Pourrut *et al*., 2008; Shahid *et al*., 2013). Mitotic index is an important indicator of cell division frequency and root growth rate. The mitotic indexes decreased with increasing Pb concentration (Table 1). High concentration Pb affected root tip cells division and prolonged interphase in cell division, thus lengthened whole cell cycle and made mitotic indexes decrease. And consequently, root growth was inhibited (Fig. 1). The mitotic indexes also decreased with prolongation of Pb treatment time. The longer Pb treatment time was, the more root tip cells were hurt.

AsA is as an ascorbate peroxidase substrate to scavenge H_2O_2 produced by ROS and plays important roles in plants under abiotic stress tolerance (Gadallah, 2000; Shigeoka *et al*., 2002). Application of AsA decreased markedly ($P < 0.05$) MN frequency under different Pb concentrations here in tested. Pb-induced MN formation may be due to potential role of oxidative stress which played a major role in damaging DNA. AsA can function as antioxidant to directly detoxify ROS. In addition, AsA may be as an enzyme cofactor to indirectly detoxify ROS. Reduction of ROS led to decrease DNA-damage induction. So, MN frequency decreased. A significant difference between application AsA and non-application AsA was also observed in CA frequency (Table 2). Dhir *et al*. (1993) reported that AsA can reduce sister-chromatid exchange caused by lead in mouse bone marrow. Exogenous application of AsA increased the protein content to NaCl-treated potatoes (Sajid and Aftab, 2009). That CA frequency decreased with exogenous application of AsA may be due to activate a complex network of proteins including of both DNA-repair proteins and antioxidant enzymes removing ROS (Wierzbicka, 1998; Balestrazzi *et al*., 2011). The exogenous application of AsA had increased the mitotic indexes than that of Pb treatment alone (Table 2). Citterio *et al*. (1994) found that AsA enhanced cell division effectiveness of competent cells. AsA content increased in pumpkin roots exposed to 50 μM aluminum sulfate stress (Dipierro *et al*., 2005). It was also reported that exogenously applied AsA enhanced *V. faba* plant seeds germination and root growth under NaCl and mannitol stress (Younis *et al*., 2010) and promoted seed germination in halophytes exposed to sea salt (Khan *et al*., 2006). In our experiment, we also found that root growth was improved with application of AsA (Data not shown). Therefore, AsA may highly alleviate genetic damage of interior cells and promote root to grow.

In conclusion, Pb genotoxicity was clearly noted on *V. faba* root cells micronucleus test in the present study. Reason of Pb genotoxicity may be due to Pb directly damaging DNA and also be due to potential role of oxidative stress. Application of AsA effectively inhibited MN and CA production and enhanced the mitotic indexes of *V. faba* root cells exposed to Pb stress. Ascorbic acid also promoted *V. faba* root growth and exerted a positive role on the *V. faba* tip cells following exposure to Pb stress. Much of the AsA is essential to resist abiotic stress during plant growth and development.

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