Bull Environ Contam Toxicol (2010) 84:161–164
DOI 10.1007/s00128-009-9914-2

Alleviation Effect of Alginate-Derived Oligosaccharides on *Vicia faba* Root Tip Cells Damaged by Cadmium

L. J. Ma · Y. Zhang · N. Bu · S. H. Wang

Received: 7 December 2008 / Accepted: 16 November 2009 / Published online: 6 December 2009

Abstract Cadmium has been shown to prevent *Vicia faba* growth by inhibiting cell mitosis. In this study we investigated the role of Alginate-derived Oligosaccharides (ADO) in alleviating *Vicia faba* root tip cells damaged by 6 and 8 mg L^{-1} $CdCl_2$. Micronucleus assay and chromosomal aberration assay were used to determine mitotic index, micronucleus frequency and chromosomal aberration frequency. The results showed that micronucleus frequency of *Vicia faba* root tip cells was inhibited under all the ADO concentrations. Especially, the inhibition ratio of 0.125% ADO highly reached 66.11 and 67.17% in 6 and 8 mg L^{-1} $CdCl_2$, respectively. Furthermore, the mitotic index increased ($p < 0.05$) and chromosomal aberration frequency decreased ($p < 0.05$) under all the ADO concentrations. This indicated that ADO had a significant alleviation effect on *Vicia faba* root tip cells damaged by cadmium.

Keywords Alginate-derived oligosaccharides · Micronucleus frequency · Mitotic index · Chromosome aberration frequency

Cadmium (Cd) is one of the most toxic trace pollutants for environment (Pinto et al. 2004). Cadmium can enter soil–plant ecology system due to the unreasonably discharge of industrial waste water, the excessive use of phosphate fertilizer and atmospheric sedimentation. Previous study showed that Cd could inhibit and prevent plant growth (Sandalio et al. 2001) by severely damaging cell structure and functions (Schützendübel and Polle 2002) and interfering with the mitosis process of plant root tip cells (He et al. 2008). It is important to develop practical techniques for alleviating the Cd stress. Exogenous application of plant growth regulators (Meng et al. 2009) and Zn (Choudhary et al. 1995) were used to alleviate Cd stress. Some species and genotypes with high Cd tolerance were developed or selected (Wu and Zhang 2004; Hassan et al. 2005; Cheng et al. 2008).

Alginate-derived oligosaccharides (M-blocks, G-blocks, ADO) are a kind of marine oligosaccharides, which are generated by alginate lyases degradation from alginate. As a resistance elicitor of plant, ADO have been resembled endogenous oligosaccharides and play an important role in agriculture due to their special physiological activities (Akimoto et al. 1999). Many researchers have identified that ADO could promote the growth of barley (Tomoda et al. 1994; Natsume et al. 1994), enhance the capacity of plant resistance for chilling, and facilitate seed germination and root elongation or promote plant produce antitoxin (Yonemoto et al. 1993; Natsume et al. 1994; Hu et al. 2004). However, the function of ADO in alleviating *Vicia faba* root tip cells damaged by Cd^{2+} has not been evaluated. Therefore, the purpose of this study is to investigate the alleviation effect of different ADO concentrations on the mitotic index, the micronucleus permillage, and chromosomal aberration of *Vicia faba* root tip cells damaged by Cd^{2+}.

Materials and Methods

The *Vicia faba* seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite and soaked in the $CdCl_2$ solutions of

L. J. Ma · Y. Zhang · N. Bu (✉) · S. H. Wang
College of Chemistry and Life Science, Shenyang Normal University, 253, Huanghe Street, 110034 Shenyang, China
e-mail: buning60@sohu.com

L. J. Ma
e-mail: malianju@tom.com

different Cd^{2+} concentrations (0, 2, 4, 6, 8 and 10 mg L^{-1}) for 24 h, and then incubated at 23°C. On the 7th day after sowing, root length was determined.

Another batch of seeds were soaked for 24 h in ADO solutions of different concentrations (1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.03125%, 0). After sufficient imbibitions, seeds were transferred in trays and cultured at 23°C. Water was changed once every 12 h. Until their length was about 1 cm, roots treated with a given ADO solution were divided into two groups. The roots of Group one were cultured in distilled water for 6 h; the roots of Group two were cultured in 6 and 8 mg L^{-1} $CdCl_2$ solution for 6 h. After that, the roots were washed in distilled water and recultured for 24 h.

The root tips were excised about 1 cm from recultured seeds during cell division crest-time. Then the root tips were soaked in Carnoy's fixation fluid (anhydrous ethanol: glacial acetic acid = 3:1, V/V) for 24 h, and kept in 70% ethanol in refrigerator at 4°C.

The root tips were first soaked in distilled water for 5 min; then decollemented in 1 M HCl for 13 min at 60°C; after HCl treatment, soaked them in distilled water for 1 min; finally stained with Feulgen.

The micronucleus frequency, cell mitotic index and chromosomal aberration frequency were examined and counted microscopically on squashes. Ten root tips were used in each treatment. SPSS was used for analysis of variance (SPSS 11.0 version).

Results and Discussion

To determine of the effect of Cd^{2+} on root length, we have checked several different Cd^{2+} concentrations (0, 2, 4, 6, 8 and 10 mg L^{-1}). The treatment with 2 mg L^{-1} Cd^{2+} resulted in the longest seedling root with a value even longer than the control (Fig. 1). The 4 mg L^{-1} Cd^{2+} treatment showed an insignificant difference with the control in term of root length. All other levels of Cd^{2+} treatment were significantly shorter in comparison with the control. The lower Cd^{2+} concentration (2 mg L^{-1}) significantly stimulated the growth of roots. However, the higher Cd^{2+} concentrations inhibited the growth of roots.

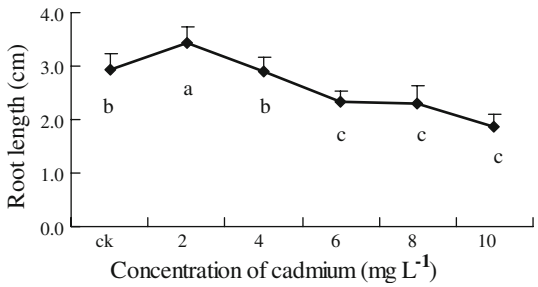

Fig. 1 Effect of Cd^{2+} on root length of *Vicia faba*

Micronucleus frequency of *Vicia faba* root tip cells induced by different ADO concentrations were apparently lower than that of the control ($p < 0.01$) (Table 1). The production of micronucleus was inhibited significantly by ADO, and inhibition ratio reached above 70% for all the ADO concentrations. Alginate-derived oligosaccharides (ADO) had a turnover dose–effect on inhibiting micronucleus frequency at 0.0625% ADO. The inhibition ratio of micronucleus frequency relatively increased when ADO concentration varied from 1 to 0.0625%, but relatively decreased after the turning point. Thus the micronucleus frequency of 0.0625% ADO treatment was the lowest ($1.12 \pm 1.03‰$, $p < 0.01$) and a maximum of inhibition ratio of 82.61% could be obtained correspondingly. This seemed that the highly concentrated solution caused the cell osmotic pressure to decrease, so that the ability of cell absorbing ADO decreased. And consequently the highly concentrated ADO had higher micronucleus frequency than that of the low concentrated ADO. When treatment was below 0.0625% ADO, micronucleus frequency increased. It seemed that ADO concentration was too low, which has less effect on *Vicia faba* root tip cells.

The mitotic indexes of *Vicia faba* root tip cells treated with different ADO concentrations were higher than that of the control (Table 1). The mitotic indexes of *Vicia faba* root tip cells increased when ADO concentration varied from 1 to 0.0625%. The mitotic indexes of 0.0625% ADO treatment was the highest, which was $6.12 \pm 2.04\%$. When treatment was below 0.0625% ADO, the mitotic indexes began to decrease. This seemed that suitable ADO concentration prolonged cell division time and shortened interphase in cell division, so that division cycle shortened.

The chromosomal aberration frequency of *Vicia faba* root tip cells treated by different ADO concentrations was remarkably lower than that of the control ($p < 0.05$) (Table 1). The chromosomal aberration frequency gradually decreased with ADO concentration varying from 1 to 0.0625%. The 0.0625% ADO treatment had the lowest chromosomal aberration frequency. Alginate-derived oligosaccharides (ADO) apparently restrained the chromosomal aberration of *Vicia faba* root tip cells.

Micronucleus frequency of *Vicia faba* root tip cells induced by 6 and 8 mg L^{-1} Cd^{2+} highly reached 26.14 ± 2.61 and $28.36 \pm 1.35‰$, respectively (Table 2). A phenomenon has been found that the root length treated by 6 and 8 mg L^{-1} Cd^{2+} shortened in compare to that of the control (Fig. 1). It seemed that 6 and 8 mg L^{-1} Cd^{2+} caused some cells chromosomal aberration and some cells mitosis were damaged and failed to enter the next cell cycle. So, the elongation of *Vicia faba* root was suppressed. Similar results have also been documented for mustard and

Table 1 Effect of different ADO concentrations on *Vicia faba* root tip cells

ADO (%)	Micronucleus frequency $\bar{x} \pm S$ (‰)	Inhibition ratio (%)	Mitotic index $\bar{x} \pm S$ (%)	Chromosome aberration frequency $\bar{x} \pm S$ (%)
Ck	6.44 ± 2.14	–	2.89 ± 1.54	11.51 ± 3.86
1	1.71 ± 1.09**	73.45	4.05 ± 1.37	6.14 ± 1.89*
0.5	1.39 ± 1.12**	78.42	4.30 ± 2.03	5.17 ± 2.51*
0.25	1.31 ± 1.23**	79.66	4.59 ± 2.19	4.78 ± 1.54*
0.125	1.22 ± 1.17**	81.06	5.28 ± 1.43*	3.59 ± 2.27*
0.0625	1.12 ± 1.03**	82.61	6.12 ± 2.04*	3.33 ± 2.18*
0.03125	1.19 ± 1.25**	81.52	5.87 ± 2.11*	3.94 ± 1.85*

Compared with the control

* $p < 0.05$; ** $p < 0.01$

Table 2 Effect of the application ADO on *Vicia faba* root tip cells treated with $CdCl_2$

ADO (%)	$CdCl_2$ (mg L^{-1})	Micronucleus frequenc $\bar{x} \pm S$ (‰)	Inhibition ratio (%)	Mitotic index $\bar{x} \pm S$ (%)	Chromosome aberration frequency $\bar{x} \pm S$ (%)
0	6	26.14 ± 2.61	–	7.28 ± 0.64	14.1 ± 2.05
1	6	16.42 ± 2.88**	37.18	6.30 ± 1.42	11.3 ± 1.81
0.5	6	13.29 ± 4.19**	50.27	7.87 ± 1.55*	10.7 ± 2.14
0.25	6	13.00 ± 3.27**	49.16	9.6 ± 2.74*	9.1 ± 1.35
0.125	6	8.86 ± 3.15**	66.11	10.56 ± 2.00*	7.7 ± 1.16*
0.0625	6	10.57 ± 2.82**	59.56	8.24 ± 1.65*	6.7 ± 2.34*
0.03125	6	14.29 ± 2.21**	45.33	7.73 ± 1.86	8.0 ± 1.96
0	8	28.36 ± 1.35		7.51 ± 2.02	15.35 ± 1.38
1	8	17.21 ± 2.42**	39.32	7.65 ± 1.13	12.01 ± 1.47
0.5	8	13.65 ± 3.25**	51.87	8.02 ± 1.37	11.26 ± 1.76
0.25	8	13.23 ± 1.17**	53.35	9.4 ± 1.87*	9.8 ± 2.14
0.125	8	9.31 ± 2.26**	67.17	9.89 ± 2.03*	8.5 ± 2.11*
0.0625	8	9.06 ± 2.53**	68.05	9.02 ± 0.94*	7.3 ± 1.96*
0.03125	8	13.76 ± 1.89**	51.48	8.37 ± 1.52	9.26 ± 1.30

Compared with only $CdCl_2$ treatment without ADO

* $p < 0.05$; ** $p < 0.01$

maize by supply of cadmium (Singh and Tewari 2003; Kumar et al. 2008). The application of the ADO decreased significantly ($p < 0.01$) the micronucleus frequency of *Vicia faba* root tip cells both 6 and 8 mg L^{-1} Cd^{2+}. The inhibition ratio of 0.125% ADO highly reached 66.11 and 67.17% in 6 and 8 mg L^{-1} Cd^{2+}, respectively. So, 0.125% ADO may inhibit the production of the micronucleus of *Vicia faba* root tip cells damaged by Cd^{2+}, which may highly alleviate genetic damage of interior cells.

The application of ADO, except for 1% ADO in 6 mg L^{-1} Cd^{2+}, had increased the mitotic indexes of *Vicia faba* root tip cells (Table 2). Especially, there was a significant increase of the 0.25, 0.125 and 0.0625% ADO treatments with the control for the mitotic indexes. This showed that ADO alleviated Cd^{2+} damage by increasing the mitotic indexes.

The chromosomal aberration frequency all decreased with the application of ADO (Table 2). The chromosomal aberration frequency treated with application of 0.125% and 0.0625% ADO were significantly lower than that of only Cd^{2+} treatment ($p < 0.05$) both 6 and 8 mg L^{-1} Cd^{2+}.

Alginate-derived oligosaccharides (ADO) effectively inhibited the production of the micronucleus and chromosomal aberration and enhanced the mitotic indexes of *Vicia faba* root cells. The results showed that ADO had a significant alleviation effect on the *Vicia faba* tip cells damaged by Cd^{2+} and showed a potential use in the bioremediation of the heavy metal damage.

Acknowledgments This work was supported by the National Natural Science Foundation of China (30870205) and the Liaoning province Natural Science Foundation (20052053).

References

Akimoto C, Aoyagi H, Tanaka H (1999) Endogenous elicitor-like effects of alginate on physiological activities of plant cells. Appl Microbiol Biotechnol 52:429–436

Cheng W, Zhang G, Yao H, Zhang H (2008) Genotypic difference of germination and early seedling growth response to Cd stress and its relation to Cd accumulation. J Plant Nutr 11:702–715

Choudhary M, Bailey LD, Grant CA, Leisle D (1995) Effect of Zn on the concentration of Cd and Zn in plant tissue of two durum wheat lines. Canadian J Plant Sci 75:445–448

Hassan MJ, Shao G, Zhang G (2005) Influence of cadmium toxicity on antioxidant enzymes activity in rice cultivars with different grain Cd accumulation. J plant Nutr 28:1259–1270

He JY, Ren YF, Zhu C, Jiang DA (2008) Effects of cadmium stress on seed germination, seedling growth and seed amylase activities in rice (Oryza sativa). Rice Sci 15(4):319–325

Hu XK, Jiang XL, Hwang Hueymin, Liu SL, Guan HS (2004) Promotive effects of alginate-derived oligosaccharides on maize seed germination. J Appl Phycol 16:73–76

Kumar P, Tewari RK, Sharma PN (2008) Cadmium enhances generation of hydrogen peroxide and amplifies activities of catalase, peroxidases and superoxide dismutase in maize. J Agrono Crop Sci 194:72–80

Meng HB, Hua SJ, Shamsi IH, Jilani G, Li YL, Jiang LX (2009) Cadmium-induced stress on the seed germination and seedling growth of *Brassica mapus* L., and its allevitation through exogenous plant growth reglators. Plant Growth Regul 58:47–59

Natsume M, Kamo Y, Hirayama M, Adachi (1994) Isolation and characterization of alginate-derived oligosaccharides with root growth-promoting activities. Carbohydr Res 258:187–197

Pinto AP, Mota AM, Varennes de, Pinto FC (2004) Influence of organic matter on the uptake of cadmium, zinc, copper and iron by Sorghum plants. Sci Total Environ 326:239–247

Sandalio LM, Dalruzo HC, Gomez M, Romero-Puetras MC, del-Rio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot 52(364):2115–2126

Schützendübel A, Polle A (2002) Plant responses to abiotic stress: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Singh PK, Tewari RK (2003) Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. J Environ Biol 24:107–112

Tomoda Y, Umemura K, Adachi T (1994) Promotion of barley root elongation under hypoxic conditions by alginate lyase–lysate. Biosci Biotechnol Biochem 58(1):202–203

Wu FB, Zhang GP (2004) Effect of cadmium on free amino acids, glutathione and ascorbic acid content in two barley genotypes differing in Cd tolerance. Chemosphere 57:447–454

Yonemoto Y, Tanaka H, Hisano T, Sakaguchi K, Abe S, Yamashita T, Kimura A, Murata K (1993) Bacterial alginate lyase gene: nucleotide sequence and molecular route for generation of alginate lyase species. J Ferment Bioeng 75:336–342