Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research article Open access

Effect of salicylic acid seed priming on resistance to high levels of cadmium in lettuce (*Lactuca sativa* L.)

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DOI: 10.31383/ga.vol2iss2pp67-72

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Received
September, 2018
Accepted
November, 2018
Published
December, 2018

Abstract

Cadmium (Cd) is an abundant heavy metal with highly toxic effects on living organisms. The aim of the presented study was to investigate the effect of seed priming using salicylic acid on cadmium tolerance in lettuce. The tolerance level was evaluated through germination speed and percentage, fresh and dry seedling weight, water and photosynthetic pigments content. Control seeds were primed with distilled water. In control plants cadmium induced reduction in germination rate, fresh and dry weight, water and chlorophyll content. Seed priming using salicylic acid, on the other hand, induced increased resistance of lettuce to cadmium toxicity. Increase in germination rate, fresh mass and water content were recorded for plants subjected to 5 mM Cd, grown from seeds primed with salicylic acid. Alleviation of Cd stress was evident through chlorophyll content as well, where smaller decrease in primed plants was recorded comparing to the control. The results obtained in this study suggest that salicylic acid can be used as priming agent in order to alleviate Cd toxicity in lettuce.

Keywords

Cadmium, heavy metal tolerance, lettuce, photosynthetic

Introduction

The provision of safe food has been one of main components of food security in the world (Zorrig et al., 2013). However, since the industrial revolution, heavy metals have been widely used in industry for different processes such as galvanisation and electro-plating. Extensive use of cadmium in these processes as well as through manufacturing of alloys, pigments and plastics, leads to soil pollution with this element (Bryne et al., 2009). It is estimated that more than 30.000 metric tonnes per year of Cd is released into the atmosphere, and having in mind that Cd does not break down in nature, human exposure to this heavy metal is constant (ATSDR, 2012). Due to high level of exposure, cadmium is listed as one of 126 priority contaminants and one of human carcinogens (IARC, 1993).

The effects of cadmium on human health include a range of neurotoxic, teratogenic, pulmonary and endocrine effects (Nordberg et al., 2007; Zorrig et al., 2013). Cultivation of plants in Cd-contaminated soil can lead to absorption of this toxic metal by edible plants which can result in contamination of food chain through trophic transfer into human diet (Zorrig et al., 2013). Lettuce is abundantly grown crop that can accumulate high levels of Cd in their leaves (Mensch & Baize, 2004). For this reason lettuce is often used as a model plant for the development of different strategies for higher Cd tolerance and decrease of Cd accumulation in plant tissues (Clemens, 2006). Priming is one of frequently used methods for seed vigour improvements in crop plants (Roychoudhury et al., 2016). Seed pre-treatments are used in order to improve seed germination and plant performance under stress conditions (Karalija & Selović, 2018). Positive effects of priming in plant resistance have been demonstrated for different crop plants such as wheat (Iqbal & Ashraf, 2007), sunflower (Kaya et al. 2006), chickpea (Kaur et al., 2002) and maize (Karalija & Selović, 2018).

It is considered that cadmium is transported through membrane transport systems into the plant cells (Takahashi et al., 2011) with likely involvement of zinc and calcium transporters in the Cd transport itself (Manohar et al., 2011; Zorrig et al., 2011). Entrance of cadmium into the plant cells triggers a wide range of toxic effects on photosynthesis through decrease in chlorophyll concentrations, induction of oxidative stress as well as imbalance in plant water status (Razinger et al., 2008; López-Millán et al., 2009). There are numerous studies regarding the Cd accumulation in lettuce as well as toxic effects of Cd on growth and other physiological parameters. However, there are no available researches regarding the effect of salicylic acid seed priming on Cd tolerance in lettuce. Thus, the goal of this research was to evaluate the effect of salicylic acid as priming agent in lettuce resistance to high Cd concentrations in regard to basic physiological and growth parameters.

Materials and methods

Seed priming and growth conditions

Lettuce seeds (*Lactuca sativa* L. variety NANSEN'S NOORDPOOL) were purchased from manufacturer Royal seeds, Italy. Healthy seeds (with no visible damages) were counted (25 seeds per petri dish), grouped according to pre-treatment (distilled water – control; 0,5 mM salicylic acid) and subsequently soaked in appropriate solution over night at 4°C for seed priming, followed by seed drying for 48 hours at room temperature. The dried seeds were stored at +4°C until cultivation.

Media for lettuce cultivation were prepared based on Murashige and Skoog (1962; MS medium) mineral salt composition, 3% (w/v) sucrose (Semikem) as carbon source and 0.8% (w/v) agar (HiMedia) as solidifying agent which was added to the media after pH adjustment to 5.8 using 10% KOH (w/v; Semikem). Media was autoclaved and transferred to sterile Petri dishes.

Lettuce seeds were cultivated in three different media types, MS medium, MS medium supplemented with 5mM $Cd(NO_3)_2$ (Semikem) and MS medium supplemented with 50 mM Cd $(NO_3)_2$. Cadmium nitrate was added to the medium prior to pH adjustment.

For each pre-treatment the total of 100 seeds (25 seeds per petri dish) were cultivated for each media type, and experiment was repeated three times (total of 300 seeds per treatment were cultivated). All the petri dishes were kept in growth room illuminated by cool white fluorescent light (5200 lm) for dark/light photoperiod of 16h at constant room temperature of 25 ± 2°C for 4 weeks.

Germination test

During the first week of culture germination speed was recorded by visually identifying and counting germinated seed every day. Germination was defined as the visible emergence of radicle through the seed coat. The germination speed coefficient (Kotowski, 1926) was calculated using the following formula:

$$GS = \frac{\sum n}{\sum Dn}$$

Where: GS - germination speed coefficient, n is the number of germinated seeds on day D, and D is the number of days from the beginning of the test.

After 4 weeks of cultivation the total germination percentage was calculated using the following formula:

$$\%G = \frac{n}{N} * 100$$

Where: %G - percentage of germination; n – number of germinated seeds; N- total number cultivated seed.

Analysis of biomass production

After four weeks of cultivation the seedlings were removed from petri dishes, the roots were washed in order to remove leftover agar and the seedlings were blotted with filter paper to remove any water excess. The seedlings were weighed to record fresh mass, dried at 70°C for 72h and weighed to record dry mass. The content of fresh and dry mass was expressed as milligrams per seedling (mg FW or DW/seedling, respectively). Biomass production was calculated using the following formula:

$$\%BM = \frac{E - C}{C} * 100$$

Where: %BM – percentage of biomass production; E is dry mass of experimental plants; C - dry mass of control plants.

The water content was calculated according to the formula (Kumari et al., 2007):

$$WC\% = \frac{Fresh\ weight - dry\ weight}{Fresh\ weight} * 100$$

Analysis of photosynthetic pigments content

Acetone extraction of pigments was obtained by maceration of 100 mg of air dried plant material with the addition of 10 mL of 100% acetone. After centrifugation supernatant was used for pigment analysis. Pigment content was evaluated spectrophotometrically (Lambda 25 spectrophotometer; Perkin-Elmer) by absorbance readings at 661.6 nm for Chl a; 644.8 nm for Chl b and 470 nm for carotenoids. Quantification of chlorophylls and carotenoids was done according to the Lichtenthaler (1987) using molar coefficients for 100 % acetone and expressed as milligrams of chlorophyll per gram of DW.

$$Chl_a(mgml^{-1}) = 11.24A_{661.6} - 2.04A_{644.8}$$

$$Chl_b(mgml^{-1}) = 20.13A_{644.8} - 4.19A_{661.6}$$

$$Chl_{a+b}(mgml^{-1}) = 7.05\ A_{661.6} + 18.09A_{644.8}$$

$$C_{car}(mgml^{-1}) = \frac{1000A_{470} - 1.90Chl_a - 63.14Chl_b}{215}$$

Statistical analysis

Experiment followed randomised design, all the treatments were represented with 4 petri dishes containing 25 seeds for each media type. The experiment was repeated three times. All the results were expressed as mean values of three replicates (± standard deviation; SD). Variance analysis was performed using ANOVA Newman-Keuls post hoc test. The differences between treatments were evaluated at $p<0.05$ level. All statistical analyses were performed using Statistica 10.0 software (Copyright© StatSoft. Inc. 1984–2011).

Results and Discussion

Exposure to cadmium decreased germination rate proportional to Cd concentration in the media. Reduction of germination rate ranged from 8% up to 40% compared to control. Salicylic acid priming alleviated the decrease in the rate of germination when seeds were exposed to 5 mM cadmium by 10% compared to the control seed lot that was exposed to 5 mM cadmium. Germination speed remained unchanged for seeds primed with salicylic acid and exposed to 0 and 5 mM cadmium. Overall germination speed was higher for seeds primed with salicylic acid compared to the control group under Cd stress (Table 1). Toxic effects of cadmium are demonstrated through the decrease in germination rate of lettuce as previously recorded (Sethy & Gosh, 2013; Bautista et al., 2013).

Reduced growth of lettuce under cadmium stress has been previously recorded even at much lower Cd concentrations (3.0 mM up to 15.0 mM; Zorrig et al., 2013). Similar growth restrictions were recorded in our research as well. However, our results indicated beneficial effects of salicylic acid priming through alleviation of toxic cadmium effects on lettuce growth. Lettuce grown in 5 mM Cd supplemented medium showed better overall growth when seeds were previously pre-treated with salicylic acid (Figure 1). Neither chlorosis nor necrosis were recorded with lower Cd concentration which was previously noted for lettuce (Zorrig et al., 2013).However, the highest Cd concentration (50 mM) induced root and shoot necrosis in all plants regardless of priming (Figure 1).

Exposure to cadmium induced reduction of fresh and

Table 1. The effect of seed priming on seed germination in lettuce

$Cd(NO_3)_2$ (mM)	Salicylic acid (mM)	Germination percentage (%)	Germination speed
0	0	80.00 [a] ±5.93	1.29 [a] ±0.09
5	0	67.20 [b] ±8.00	1.12 [b] ±0.13
50	0	40.00 [d] ±5.21	0.67 [d] ±0.08
0	0.5	72.80 [ab] ±3.57	1.21 [ab] ±0.06
5	0.5	72.00 [ab] ±3.34	1.20 [ab] ±0.05
50	0.5	44.00 [c] ±5.65	0.85 [c] ±0.09

Data represent means of three replicates (± SD). Values within one parameter followed by the same letter do not differ significantly after ANOVA post hoc Newman-Keulstest at significance level of $p<0.05$

dry weight per seedling in concentration dependent manner. Seed priming with 0.5 mM salicylic acid induced rise in fresh mass due to higher water content. Exposure to cadmium induces a decrease in water uptake by suppressing the short distance water transport through symplast and apoplast resulting in drought stress in plants (Rucińska-Sobkowiak, 2016). Pre-treatment of lettuce seeds with salicylic acid induced higher water uptake and resulted in higher water content in seedlings. Dry mass of seedlings did not differ significantly between control and salicylic acid pre-treated seeds under equal Cd exposure (Table 2). Due to high necrosis of seedlings cultivated with Cd concentration that is extremely toxic for plants (50 mM) the analysis of photosynthetic pigments for this treatment was not performed.

Table 2. The effect of seed priming on fresh and dry weight content in lettuce seedlings

$Cd(NO_3)_2$ (mM)	Salicylic acid (mM)	Fresh weight per seedling (mg)	Dry weight per seedling (mg)	Water content (%)
0	0	68.35 [b] ±11.28	4.36 [a] ±0.34	93.62 ±0.57
5	0	58.30 [b] ±5.94	3.56 [b] ±0.26	93.89 ±0.17
50	0	10.61 [c] ±0.40	1.92 [c] ±0.17	81.90 ±0.95
0	0.5	102.6 [a] ±12.56	4.41 [a] ±0.17	95.70 ±0.36
5	0.5	96.90 [a] ±5.58	3.54 [b] ±0.07	96.34 ±0.13
50	0.5	10.30 [c] ±0.55	1.64 [c] ±0.06	84.07 ±0.27

Data represent means of three replicates (± SD). Values within one parameter followed by the same letter do not differ significantly after ANOVA post hoc Newman-Keuls test at significance level of $p<0.05$.

Overall, seed priming with salicylic acid induced decrease in photosynthetic pigment content when plants were not subjected to cadmium induced stress. The exposure to cadmium induced drastic drop in chlorophyll content in control plants, while in plants of salicylic acid pre-treated seeds photosynthetic pigment content did not differ significantly between treatments (Table 3). The reduction of chlorophyll *a* content under Cd exposure due to cadmium induced inhibition of chlorophyll biosynthesis (Hsu & Kao, 2003) has been previously recorded (Liu et al., 2014). Higher Cd concentrations induce the degradation of chlorophyll *b* as well (Liu et al., 2014) which is also

Table 3. Effect of seed priming on photosynthetic pigments content in lettuce seedlings

$Cd(NO_3)_2$ (mM)	Salicylic acid (mM)	Chlorophyll *a* (mg/g DW)	Chlorophyll *b* (mg/g DW)	Total chlorophylls (mg/g DW)	Carotenoids (mg/g DW)	Chlorophyll a/chlorophyll b
0	0	3.596 [a] ±0.241	2.882 [a] ±0.450	6.477 [a] ±0.691	0.554 [b] ±0.012	1.25 ±0.12
5	0	2.956 [a] ±0.426	1.808 [b] ±0.488	4.764 [b] ±0.911	0.640 [a] ±0.056	1.63 ±0.25
0	0.5	1.466 [b] ±0.817	1.544 [b] ±0.771	3.464 [bc] ±1.573	0.359 [c] ±0.064	0.95 ±0.28
5	0.5	1.561 [b] ±0.006	1.088 [b] ±0.056	2.649 [c] ±0.053	0.332 [c] ±0.012	1.45 ±0.07

Data represent means of three replicates (± SD). Values within one parameter followed by the same letter do not differ significantly after ANOVA post hoc Newman-Keuls test at significance level of $p < 0.05$.

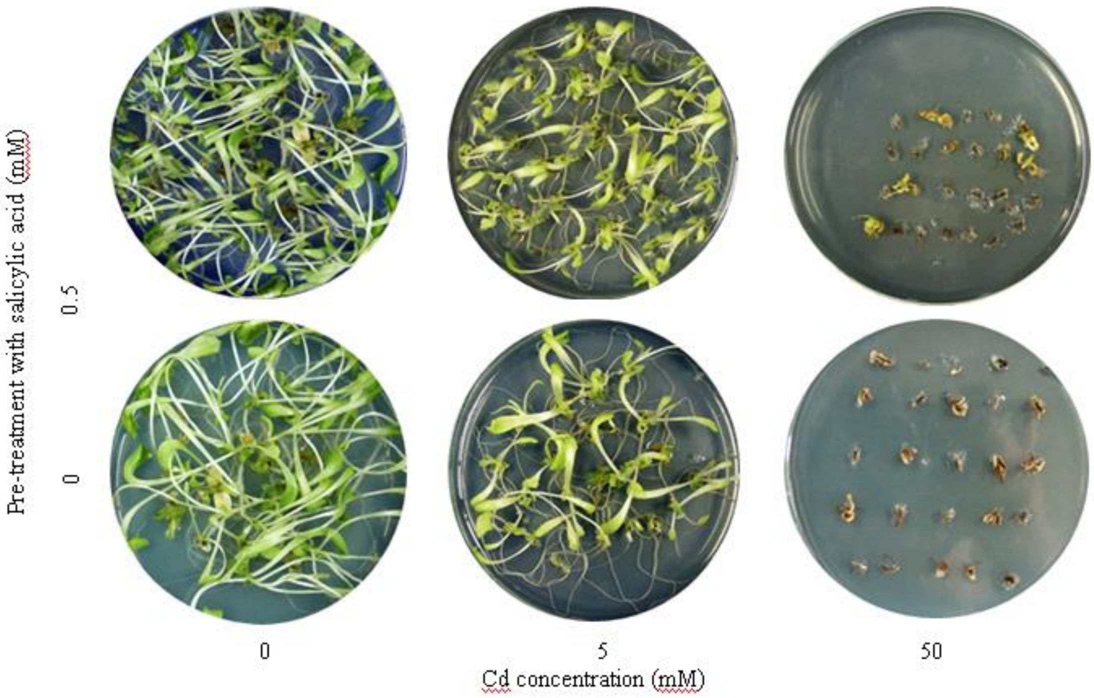

Figure 1. Effect of seed priming on plant growth in relation to cadmium concentration in the media

noted in our study for control plants. Pre-treatment of seeds with salicylic acid induced an increase of chlorophyll *b* in the medium without Cd. Exposure to cadmium induced degradation of chlorophyll *b* in these plants as well and slight increase of chlorophyll *a* content resulting in higher Chl *a*/*b* ratio (Table 3). Similar increase of Chl *a*/*b* ratio under Cd stress as a result of chlorophyll *b* breakdown was previously recorded in cotton (Liu et al., 2014).

Conclusions

Application of salicylic acid as a priming agent can induce higher resistance to cadmium stress in lettuce as demonstrated in this study. Increased tolerance is demonstrated through higher germination rate, fresh mass, and water content under heavy metal stress conditions. Our results suggest that cadmium acts as major limiting factor for plant growth but its toxicity can be alleviated through seed priming using salicylic acid.

References

ATSDR (2012) *Toxicological profile for cadmium*. Agency for toxic substance and disease registry. US Department of Health and Humans Services, Public Health Service, Canter for Disease Control, Atlanta, GA, USA, 487.

Bautista OV, Fischer G, Cárdenas, JF (2013) Cadmium and chromium effects on seed germination and root elongation in lettuce, spinach and Swiss chard. Agron Colomb, 31:48-57.

Byrne C, Divekar SD, Storchan GB, Parodi A, Martin MB (2009) Cadmium – a metallohormone? Toxicol Appl Pharmacol, 238:266-271.

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie, 88**:**1707-1719.

Hsu YT, Kao CH (2003) Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. Plant Cell Environ, 26:867-874.

IARC (1993) Monographs on the evaluation of carcinogenic risks to humans, beryllium, cadmium, mercury, and exposures in the glass manufacturing industry.IARC, Lyon, France. 58:239-345.

Iqbal M, Ashraf M (2007) Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. J Integr Plant Biol, 49:1003-1015.

Karalija E, Selović A (2018) The effect of hydro and proline seed priming on growth, proline and sugar content, and antioxidant activity of maize under cadmium stress. Environ Sci Pollut Res, 25(33):33370-33380.

Kaur S, Gupta AK, Kaur N (2002) Effect of osmo-and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. Plant Growth Regul, 37:17-22.

Kaya MD, Okçu G, Atak M, Çıkılı Y, Kolsarıcı Ö (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur J Agron, 24:291-295.

Kotowski F (1926) Temperature relations to germination of vegetable seeds. Proc Amer Sot Hort, 23:176-184.

Kumari J, Gadag RN, Jha GK (2007) Genetic analysis and correlation in sweet corn (*Zea mays*) for quality traits, field emergence and grain yield. Indian J Agr Sci, 77(9):63-65.

Lichtenthalter H (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol, 148:350-382.

Liu L, Sun H, Chen J, Zhang Y, Li D, Li, C (2014) Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton (*Gossypium hirsutum* L.). Plant Omics, 7:284.

López-Millán AF, Sagardoy R, Solanas M, Abadia A, Abadia J (2009) Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. Environ Exper Bot, 65**:**376-385.

Manohar M, Shigaki T, Hirschi KD (2011) Plant cation/H+ exchangers (CAXs): Biological functions and genetic manipulations. Plant Biol, 13:561-569.

Mensch M, Baize, D (2004) Contamination des sols et de nos aliments d'originevégétale par les élémentsen trace, mesures pour réduirel'exposition. Courrier de l'Environnementdel'INRA, 52:31-54.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15:473-497.

Nordberg GF, Fowler BA, Nordberg M, Friberg L (2007) *Handbook on the Toxicology of Metals*. 3rd Edition, Academic Press, New York, 445-486.

Razinger J, Dermastia M, Koce JD, Zrimec A (2008) Oxidative stress in duckweed (*Lemna minor* L.) caused by short-term cadmium exposure. Environ Pollut, 153:687-694.

Roychoudhury A, Ghosh S, Paul S, Mazumdar S, Das G, Das S (2016) Pre-treatment of seeds with salicylic acid attenuates cadmium chloride-induced oxidative damages in the seedlings of mung bean (*Vignara diata* L. Wilczek). Acta Physiol Plant, 38:11.

Rucińska-Sobkowiak, R (2016) Water relations in plants subjected to heavy metal stresses. Acta Physiol Plant, 38:257.

Sethy SK, Ghosh S (2013) Effect of heavy metals on germination of seeds. J Nat Sc Biol Med, 4:272-275.

Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa, NK (2011) Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. Plant Signal Behav, 6**:**1813-1816.

Zorrig W, Abdelly C, Berthomieu P (2011) The phylogenetic tree gathering the plant Zn/Cd/Pb/Co P1B-ATPases appears to be structured according to the botanical families. C R Biol, 334**:**863-871.

Zorrig W, El Khouni A, Ghnaya T, Davidian JC, Abdelly C, Berthomieu P (2013) Lettuce (*Lactuca sativa*): a species with a high capacity for cadmium (Cd) accumulation and growth stimulation in the presence of low Cd concentrations. J Hortic Sci Biotechnol, 88:783-789.