Screening of Watermelon Varieties for Lead Tolerance at the Seedling Stage

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Abstract. Watermelon (*Citrullus lanatus*) is an important horticultural crop that is sensitive to heavy metals such as lead (Pb) in polluted water or soil. However, there are no available data regarding Pb tolerance phenotyping in watermelon. Watermelon seedlings were exposed to various Pb doses (0, 20, 40, 60, 80 $\mu\text{M}\cdot\text{L}^{-1}$ Pb) for 14 days, after which 20 μM Pb was identified as the optimal treatment for lead tolerance analysis in watermelon because it caused significant symptoms (leaf chlorosis, stubby and yellow roots) but little damage to seedlings. Subsequently, the Pb responses were analyzed in eight watermelon varieties (V1–V8), and membership function analysis was used to determine a single Pb tolerance index. Of the eight watermelon varieties, V4 and V7 were ranked the most Pb tolerant; V1, V2, V5, and V6 were moderately Pb tolerant; and V3 and V8 were the most Pb-sensitive varieties. Compared with most Pb-sensitive varieties (V3 and V8), the most Pb-tolerant varieties (V4 and V7) maintained high antioxidant activity, and had lower malondialdehyde (MDA) and total soluble protein (TSP) contents. In addition, carotenoid and chlorophyll (both a and b) contents were stimulated and inhibited, respectively, in leaves of high-Pb translocation varieties (V4 and V8). Principal component analysis (PCA) revealed relative root length as an indicator of Pb tolerance because it correlated significantly with shoot growth. These results provide useful insight into the mechanism of Pb tolerance in cucurbit crops, as well as information regarding the breeding of watermelon with enhanced tolerance to this heavy metal (Pb).

Lead (plumbum, Pb) is a nonessential element for plants and one of the hazardous heavy-metal pollutants in the environment. The most important sources of Pb contamination are mining, pesticides, fossil fuels, sewage sludge, and fertilizers (Huang et al., 2017). Toxicity of Pb in plant tissues can be recognized by the alterations in biochemical and physiological processes (Maodzeka et al., 2017) associated with depression in plant growth (Zhou et al., 2014), and reduction in yield and quality (Ashraf et al., 2017; Rao et al., 2018; Zhong et al., 2017). However, the extent of these effects varies and depends on the Pb concentration and the duration of exposure (Nas and Ali, 2018; Pidatala et al., 2016), the intensity of plant stress, the stage of plant development, and the particular organs or organelles (Sun and Luo, 2018). Plants have developed various methods for responding to toxic effects of Pb exposure, such as selective uptake, binding to root surface and cell wall, and induction of antioxidants (Khan et al., 2014; Zhong et al., 2017).

Pb availability to plants depends on many factors, such as soil texture, organic matter content, pH, and the forms and concentration of Pb in the soil profile (Cândido et al., 2020; Chen et al., 2015; Radziemska et al., 2020). Plant roots have the ability to absorb significant amounts of Pb because of the thin cell walls of the root apices (Seregin et al., 2004) that greatly restrict its translocation to above-ground parts (Almasi et al., 2019). However, a greater concentration of Pb damage plasmalemma and a greater amount of it enter the cell (Jiang et al., 2018; Seregin et al., 2004). The low translocation of Pb from roots to shoots is a result of the accumulation of Pb in the intercellular spaces as insoluble phosphates, oxalates, and chlorates and, its precipitation as Pb carbonate deposited in the cell wall. Similarly, casparian strips of the endodermis restrict Pb transport into the central cylinder tissue, resulting in lower translocation to aboveground organs of the plants (Ashraf et al., 2020; Zhou et al., 2018). Despite the ability of roots to restrict Pb upward translocation, there are hyperaccumulators plants such as *Chenopodium murale* L. (Sidhu et al., 2018) and *Emilia sonchifolia* L. (Zhou et al., 2015) that accumulate greater Pb amounts in shoots.

Pb tolerance varies among different plant species (Cândido et al., 2020) and among varieties within the same species (Wang et al., 2014). Similarly, Pb uptake and accumulation varies among genotypes and cultivars of the same species (Ashraf et al., 2020; Liu et al., 2009, 2013; Maodzeka et al., 2017). These variations in Pb tolerance, uptake, and accumulation are a result of the differences in strategies used by plants to handle Pb stress, such as Pb uptake, accumulation, and translocation potential (Ashraf et al., 2020; Ho et al., 2008; Liu et al., 2013); restriction of Pb to the cell wall (Qiao et al., 2015); sequestration of Pb in vacuoles (He et al., 2015; Morel et al., 2009); synthesis and secretion of organic acids, which in turn precipitate Pb (Yang et al., 2000); and antioxidant enzyme activities (Mahdavian et al., 2016). In general, seed germination and

early stages of seedling growth are more sensitive to Pb stress and are widely used in the assessment of Pb toxicity (González-Valdez et al., 2015; Kong et al., 2014). Differences in morphological, physiological, and biochemical responses of plants to Pb stress have been used to evaluate Pb tolerance and sensitivity (Mahdavian et al., 2016; Maodzeka et al., 2017). Similarly, growth parameters such as biomass, and root and shoot length have been used as indicators of heavy-metal toxicity in plants (Alaboudi et al., 2018; Liu et al., 2020).

China is the world leading producer and consumer of watermelon, one of the most important crops worldwide (Sheng et al., 2012). Pb-contaminated agricultural soils is becoming a serious problem in watermelon production recently, especially in the main producing areas of the southeast provinces (Ashraf et al., 2020; Ma et al., 2015; Wei and Yang, 2010). Effects of Pb on watermelon growth is prominent but not fully recognized (Akinci and Çalışkan, 2010). However, it is well-known that watermelon growth may be affected seriously by the concentration of Pb in soil. Toxicity of Pb may result in symptoms such as wilting, slow growth rate, reduced fruit size, and more. We identified Pb-tolerant and -sensitive watermelon varieties, and contribute our results to the Pb-tolerant breeding of watermelon that produce more healthy fruit under Pb pollution. Therefore, we addressed for the first time Pb stress adaptation in watermelon, screened eight varieties with respect to Pb stress, and classified their tolerance based on morphological and biochemical changes. The description and clarification of Pb stress phenotyping and adaptation in watermelon could provide clues to understanding and improving Pb tolerance in other crop plants.

Materials and Methods

Plant material and hydroponic culture. Eight varieties of watermelon (*Citrullus lanatus*)—JBT, KB948, LF, NBT, XF1, XF, ZM5, and ZJ (represented as V1–V8)—were used in this study. Watermelon seeds were dipped in water at 55 °C for 15 min and then stored at room temperature for 4 h. Next, they were sterilized by 70% ethanol for 30 s and rinsed in sterile distilled water five times. The seeds were germinated at 30 °C for 24 h in darkness. Seedlings were supplied with half-strength Hoagland's solution in a growth room under control conditions (16 h/26 °C day and 8 h/23 °C night, at a light intensity of 250–300 $\mu\text{mol photon/m}^2/\text{s}$, and a relative humidity of $\approx 55\%$ to 60%). After 8 d, seedlings with uniform size were selected and wrapped in foam, and transferred into plate holes on plastic pots (five seedling per pot) containing half-strength Hoagland nutrient solution (Arnon and Hoagland, 1940), in which the monopotassium phosphate concentration was adjusted to 0.04 μM to avoid possible Pb precipitation (Zhivotovsky et al., 2010) (Supplemental Table 1). The nutrient solutions were continuously aerated and renewed every 2 d.

Dose optimization. To determine the optimal Pb concentration that causes minimal damage to watermelon seedlings, the most popular variety—ZJ—was selected. Fourteen-day-old seedlings were exposed for 2 weeks (this during was found to be nonlethal, but resulted in significant toxicity symptoms in all preexperiments) to Pb stress (0, 40, 60, 80 μM L^{-1} Pb). C1 is general control while modified control C2 and the treatment solutions containing 0.04 μM phosphorus (P) to evade possible Pb precipitation with P in the nutrient solution (Zhivotovsky et al., 2010).

Pb tolerance screening in watermelon. The root length and shoot height of each seedling were measured before and after treatment. Fresh weight of roots and shoots were weighted immediately after harvest, and were then placed in a preheated oven at 80 °C for 2 d, after which dry weight was determined using an electronic balance. The leaf area of the first fully expanded leaf was calculated using Image J software (Image J version ij152-win-java8; National Institutes of Health, Bethesda, MD). Data were collected as the mean of five similar seedlings, and relative growth was calculated as the ratio of Pb treatment to the control.

The Pb tolerance index of the watermelon varieties was calculated using the membership function by integrating the relative root length (RRL), relative shoot height (RSH), relative root fresh weight (RRFW), relative root dry weight (RRDW), relative shoot fresh weight (RSFW), relative shoot dry weight (RSDW), and relative leaf area (RLA). The Pb tolerance index was computed using Eq. [1],

$$X_u = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad [1]$$

where X_u is the subordinate value representing RRL, RSH, RRFW, RRDW, RSFW,

RSDW, and RLA. X is the measured value of the growth parameter; $X_{\max}$ and $X_{\min}$ are the maximum and minimum value of the growth parameter.

Pb content analysis and translocation factor. For elemental analysis, the two most Pb-tolerant (V4 and V7), two more Pb-tolerant (V2 and V6), and two most Pb-sensitive (V3 and V8) varieties were selected and grown in 0 and 20 μM L^{-1} Pb for 2 weeks. Treated plants were then harvested and washed with distilled water to remove impurities, and blotted dry. Roots and shoots were dried in an oven at 80 °C for 48 h, and were then ground into powder. Root and shoot samples (0.1 g) were treated sequentially in 6 mL concentrated nitric acid and 200 μL hydrogen peroxide (H_2O_2 ; 30%), and the mixture was heated using a microwave reaction system (Multiwave 3000 Anlou Paor). Thereafter, samples were digested at 160 °C for 4 h, and then diluted with double-distilled water up to 30 mL. Pb content was eventually evaluated via inductively coupled plasma optical emission spectroscopy from a diluted solution (ICP-OES optima 8000 DV; Perkin Elmer).

The translocation factor (TF) is the ability of a plant to translocate the accumulated Pb from its roots to aboveground parts. It can be calculated as shown in Eq. [2],

$$TF = \frac{\text{Pb}_{(\text{aboveground parts})}}{\text{Pb}_{(\text{roots})}} \quad [2]$$

where Pb (aboveground parts) is the concentration of Pb in aerial tissues, and Pb (roots) is the concentration of Pb in the roots.

Biochemical assays. The lipid peroxide levels were determined by measuring the MDA content from the thiobarbituric acid reaction as described by El-Moshaty et al. (1993). Samples (≈ 0.5 g) were homogenized in 1.5 mL 5% trichloroacetic acid (w/v) and centrifuged at 3000 rpm for 10 min, and then the supernatant was diluted to 10 mL. Two milliliters of the diluted extract was mixed with 2 mL 0.67% 2-thiobarbituric acid and the mixture was incubated in boiling water (100 °C) for 20 min, cooled on ice, and then centrifuged at 3000 rpm for 10 min. The absorbance was measured at 532 nm.

The measurement of TSP content was done by the homogenization of a 0.5-g (roots and leaves) sample with pestle and mortar (Bradford, 1976). The homogenate was centrifuged for 10 min at 12,000 g_n , and an aliquot of the extract was used to determine protein content, using bovine serum albumin as the standard.

Total soluble sugar (TSS) was estimated using the method of Yemm and Willis (1954) with a 0.1-g plant sample (roots and leaves). The homogenized sample was extracted twice in 80% ethanol and evaporated to dryness in a test tube in a hot water bath at 100 °C. Absorbance of this solution was

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measured at 620 nm against the reagent blank using a spectrophotometer (UV-VIS 2550; Shimadzu, Kyoto, Japan).

A 0.5-g fresh leaf sample was taken and homogenized (using a pestle and mortar) and, along with 10 mL 80% acetone, centrifuged at 10,000 rpm for 15 min at 4 °C. The solution was analyzed for chlorophyll a, chlorophyll b, and carotenoid content using a spectrophotometer (UV-VIS 2550; Shimadzu) according to Lichtenthaler and Wellburn (1983).

For the enzyme activity assay, 0.5-g plant samples (roots and leaves) were homogenized in 8 mL 50 mM potassium phosphate buffer (pH 7.8) using a chilled mortar and pestle in an ice bath. The homogenate was centrifuged at 10,000 g_n at 4 °C for 20 min and the supernatant was used to determine the enzymatic activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), as described by Mahdavian et al. (2016). All spectrophotometric analyses were conducted using a Shimadzu UV-2550 spectrophotometer.

Determination of Pb tolerance indicator parameter and statistical analysis. PCA was conducted on the relative growth parameters (RRL, RSH, RRFW, RRDW, RSW, RSDW, and RLA) to determine the major parameter causing variability among the components. The extracted components (PC1 and PC2) were represented in a scatterplot (not shown) to analyze associations among relative growth parameters based on their loading factor values. The experiment was conducted in a completely randomized design with three replicates, and three pots were used for each variety. The data were analyzed using SPSS software (SPSS Version 16.0; SPSS Inc., Chicago, IL). Differences among groups were assessed by one-way analysis of variance followed by *t* tests. Values in the text are mean $\pm$ SD (SD of three replicate samples). Differences with *P* < 0.05 were considered to be statistically significant.

Results

Effect of different Pb concentrations on watermelon seedling growth. Pb concentrations ranging from 0 to 80 μM were used to identify the optimum Pb concentration, which can cause visible symptom and minimum growth reduction in watermelon seedlings. The shoot height, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight were measured after being treated for 2 weeks with Pb at a dose of 0, 20, 40, 60, or 80 μM (Fig. 1). Significant reductions in shoot dry weight and root fresh weight were observed in C2 compared with C1 (19.9% and 20.97%, respectively), whereas other growth parameters were found to be nonsignificant and no significant differences between C1 and C2 were identified (Fig. 2C and F). In general, Pb treatment inhibited most of the growth parameters

Fig. 1. Growth comparison of watermelon variety ZJ exposed to 20 μM Pb for 14 d in Hoagland's nutrient solution. (A) Comparison showing growth of ZJ in control (C1 and C2) and in 20-, 40-, 60-, and 80- μM Pb treatments (from left to right). (B) Whole seedling in control (C1 and C2) and in 20-, 40-, 60-, and 80- μM Pb treatments (from left to right). C1 is the general control whereas C2 is the modified control (containing 0.04 μM P to avoid Pb precipitation with P in nutrient solution).

(shoot height, shoot fresh weight, shoot dry weight, root length, root fresh weight) in a dose-dependent manner (Fig. 2A–D and F), with the exception of the 20- μM Pb treatment, which increased root dry weight by 10.39% compared with control groups, suggesting that a low concentration of Pb may induce biomass accumulation in watermelon root (Fig. 2E). The greater Pb concentration (80 μM) inhibited shoot height, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight by 34.61%, 63.94%, 65.58%, 30.55%, 70.44%, and 66.66%, respectively. Thus, treatment of 20 μM Pb and a 2-week exposure was selected as the optimum condition for inducing Pb toxicity and studying Pb tolerance in watermelon, because plants started to exhibit significant differences in each parameter under this condition.

Pb tolerance index. To identify Pb-tolerant and -sensitive genotypes, eight watermelon varieties were used, and their relative growth parameters were measured 14 d after treatment with or without 20 μM Pb application. Pb tolerance based on the relative root length revealed that the majority of watermelon varieties were Pb sensitive and showed more than 50% root length inhibition except for V7 (26.33%), V1 (35.51%), and V5 (40.03%) (Fig. 3A). Pb tolerance based on the relative root fresh weight revealed that, in V4, V5, and V7, it was enhanced by 13.28%, 37.83%, and 38.07% (Fig. 3C). Pb tolerance based on the relative root dry weight revealed that it was decreased in V3 and V1 by 18.08% and 35.58%, respectively, and enhanced in other varieties (ranging from 14.2% to 239.6%) (Fig. 3D). Shoot height was not very sensitive to Pb stress, as severe inhibition was observed in V8 only (40.62%), whereas stimulated shoot height was found in V5 (4.79%) and V4 (13.24%) (Fig. 3B). However, shoot fresh weight and dry weight data did not

always correlate with the pattern of shoot height. Pb increased relative shoot fresh weight in V7 only (16.23%), whereas an inhibitory effect was found in other varieties (ranging from 4.73% in V4 to 40.43% in V3) (Fig. 3E). Pb stimulated relative shoot dry weight in V4, V1, and V7, whereas inhibition in other varieties occurred, showing other possible Pb-tolerance patterns (Fig. 3F). Similarly, leaf area was stimulated by Pb treatment in V7, V2, V4, and V6, and decreased in other varieties (Fig. 3G).

To identify the most Pb-tolerant and -sensitive watermelon varieties for further investigation, different relative growth indexes were integrated to get a single Pb tolerance index by membership function analysis (MFA) (Fig. 4). The varieties were grouped with respect to the tolerance index, and V4 and V7 were designated as the most tolerant varieties according to this grading criterion of mean value $\bar{X}_u \geq 0.8$. Four varieties (V1, V2, V5, and V6) with an $\bar{X}_u$ value around 0.4 to 0.6 were classified as moderate varieties. V3 and V8 ($\bar{X}_u \leq 0.2$) were found to be the most sensitive varieties (Fig. 4). MFA identified the most Pb-tolerant (V4 and V7) and most Pb-sensitive (V3 and V8) varieties, although it was difficult from the relative growth indexes to find the most Pb-tolerant and -sensitive varieties because of variations in trends (Fig. 3).

Pb content and its translocation in watermelon tissue. For the comparison of variations in Pb concentration and translocation factor values, the two most Pb-tolerant and two most Pb-sensitive watermelon varieties were examined. Pb concentration, accumulation, and translocation ratio varied among the watermelon varieties (Fig. 5). Pb concentration in roots was much greater than that in shoots for each variety. Interestingly, the greatest Pb concentration in both roots and shoots was found in V4 (Fig. 5A and B), which show a significantly lower Pb concentration in leaves (Fig. 5C). V8, one of the most sensitive watermelon varieties, and showed

Fig. 2. Average growth responses of roots and shoots of watermelon variety ZJ after 14 d of exposure to different concentrations of Pb in Hoagland's nutrient solution. (A) Shoot height. (B) Root length. (C) Shoot dry weight. (D) Shoot fresh weight. (E) Root dry weight. (F) Root fresh weight. Values given are mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($P \leq 0.05$) among treatments.

a much greater translocation ability than the other varieties (Fig. 5D). These results suggest that Pb tolerance and translocation are different traits.

Influence of Pb toxicity on the contents of MDA, TSP, TSS, and photosynthetic pigments. MDA content assay was used to assess the oxidative lipid damage in watermelon varieties under Pb stress. A significant increase in MDA was observed in both leaves and root tissues of the Pb-sensitive varieties, whereas it was slightly induced in tolerant watermelon varieties (Fig. 6A and B). Pb toxicity led to an opposite variation in TSP content in leaves of tolerant and sensitive varieties, even when it induced TSP accumulation in roots of both watermelon

types (Figs. 6C and 7D). TSS content was significantly increased in all varieties (Fig. 6E and F). Under Pb stress, chlorophyll a content increased in V3 and V7, whereas it declined in V8 and V4 (Fig. 7A). A similar pattern was also observed in chlorophyll b content (Fig. 7B). Contrary to chlorophyll a and b contents, carotenoid content increased in V4 (557%) and V8 (125%), but decreased by 49% in V3 (Fig. 7C). These results demonstrate that more Pb accumulation in the shoots of V4 and V8 likely disturbed the photosynthetic machinery.

Antioxidative response of watermelon varieties to Pb stress. Pb treatment affected antioxidant activities differently in the

Pb-tolerant and -sensitive watermelon varieties in terms of SOD, POD, CAT, and APX (Fig. 8). A significant increase in SOD was found in both leaves and roots of tolerant watermelons (V4 and V7; Fig. 8A and B), and slightly increased or decreased SOD was observed in less-sensitive watermelon (V8 and V3; Fig. 8A and B). Similarly, SOD activity increased in roots by 81%, 46%, and 11% in V4, V7, and V8, respectively, with a slight decrease of 0.9% in V3 (Fig. 8B). POD activity declined by 18.9% in V3 leaves, but increased enormously in the other varieties by 374%, 163%, and 43% in V7, V4, and V8 (Fig. 8C). POD activity increased in roots of V7, V4, V8, and V3 by 181%, 87%, 58%, and 45% respectively

Fig. 3. The relative growth of the root and shoot of eight watermelon varieties after 14 d of exposure to 20 μ M Pb in Hoagland's nutrient solution. (A) Relative root length. (B) Relative shoot height. (C) Relative root fresh weight. (D) Relative root dry weight. (E) Relative shoot fresh weight. (F) Relative shoot dry weight. (G) Relative leaf area. Values given are mean $\pm$ SD (n = 3). Values with different letters are significantly different ($P \leq 0.05$) among treatments.

(Fig. 8D). CAT activity increased in all varieties in both leaves and roots. The greatest CAT activity in leaves and roots was recorded for V4 (724%) and V7 (125%) (Fig. 8E and F). APX activity increased slightly and decreased in Pb-tolerant

and -sensitive watermelon varieties, respectively (Fig. 8G and H).

Determination of Pb tolerance indicator parameters in watermelon. To determine Pb tolerance indicator parameters in watermelon varieties under Pb stress, PCA of

different relative growth parameters was conducted to identify those that accounted for major component variability (Fig. 9A and B). PCA revealed two components that accounted for 67.03% of the total variance. On one hand, PC1 accounted for 43.47%

of the explained variance, which is shown in Eq. (3):

$$\begin{aligned} \text{PC1} = & 0.937 \text{RRL} + 0.896 \text{RSDW} \\ & + 0.792 \text{RSFW} - 0.341 \text{RRDW} \\ & + 0.311 \text{RRFW} - 0.062 \text{RLA} \\ & + 0.05 \text{RSL} \end{aligned} \quad [3]$$

The second component (PC2) accounted for 23.56% of the total variance, which is shown in Eq. [4]:

$$\begin{aligned} \text{PC2} = & -0.92 \text{RRL} + 0.362 \text{RSDW} \\ & + 0.366 \text{RSFW} + 0.195 \text{RRDW} \\ & + 0.821 \text{RRFW} + 0.771 \text{RLA} \\ & + 0.767 \text{RSL} \end{aligned} \quad [4]$$

This analysis identified RRL, RSDW, and RSFW (0.937, 0.896, and 0.792, respectively) as the main growth parameters contributing to the maximum principal component variation [as demonstrated in Eq. (3)]. Similarly, a significant correlation ($P < 0.01$) between RRL, RSFW, and RSDW was observed,

Fig. 4. Pb tolerance index of eight watermelon varieties after 14 d of exposure to 20 μM Pb in Hoagland's nutrient solution under greenhouse conditions.

indicating that root growth affected shoot growth positively. These results reveal that better root growth under Pb stress is predictive of better shoot growth.

Discussion

Pb contamination in water, air, and soil is a global environmental issue. Plants exhibit various toxicity symptoms to Pb exposure, including stunted growth, a decrease in biomass production, and inhibition of root and shoot growth (Hattab et al., 2016; Kumar and Prasad, 2018). Watermelon is an important horticultural crop that is sensitive to Pb stress (Osei-Agyeman, 2017; Torki et al., 2018). Morphological screening of Pb-tolerant watermelon varieties that translocate less Pb to aboveground parts will enhance our understanding of the Pb tolerance mechanism, which is as yet unknown.

After treatment with different Pb concentrations, the root dry weight of watermelon variety ZJ decreased with increasing Pb concentration in the hydroponic solution. However, the lower dose (20 μM) of Pb increased root dry weight by 10.39% in comparison with control plants (Fig. 2E), which might be a result of the increased synthesis of polysaccharides in the cell walls after exposure to Pb (Sharma and Dubey, 2005). Similarly, dry weight of radish root (Ahmad et al., 2018), as well as corn seedlings (Małkowski et al., 2002), has also been enhanced by Pb. In spinach, an increase in root dry weight was noted at 4.83 mM Pb (Khan et al., 2016). The reduction in root length was more pronounced at both lower and higher doses of Pb (20 and 80 μM) in comparison with shoot height. Our results are in agreement with those observed for *Medicago sativa* (Hattab et al., 2016), *Acalypha indica* (Venkatachalam et al., 2017), and *Thespesia populnea* (Kabir et al., 2008). Because

plant response to Pb stress presents huge variations depending on genotype and tolerance mechanisms (Huang et al., 2017; Maodzeka et al., 2017), new statistical approaches are required to quantify Pb tolerance in watermelon via phenotype. In our study, 20 μM Pb was identified as the optimum dose to induce Pb toxicity, and MFA was used to classify Pb tolerance in watermelon. For heavy-metal tolerance quantification, MFA has been widely used in other plants such as wheat (Ci et al., 2011; Shi et al., 2015), sunflower (Ma et al., 2016), and tobacco (Maodzeka et al., 2017).

Pb accumulation was analyzed in the roots and shoots of different tolerant watermelon varieties, and diverse levels of Pb were found, which did not seem to correlate with Pb tolerance (Fig. 5). In all tested varieties, roots accumulated much more Pb than shoots. Similar results have been reported previously in lettuce (Liao et al., 2006), tobacco (Maodzeka et al., 2017), *Salix integra* (Wang et al., 2014), and *Hibiscus cannabinus* (Ho et al., 2008). This phenomenon might result from inhibited element upward transportation. Interestingly, significant differences were observed in shoot Pb accumulation of the two most Pb-tolerant varieties: V4 accumulated more than twice the Pb than that found in V7. Similarly, the most Pb-sensitive V8 accumulated more Pb than in another most Pb-sensitive watermelon (Fig. 5B). However, the sensitive varieties accumulated significantly greater amount of Pb in leaves than the tolerant varieties (Fig. 5C). The translocation factor values of all watermelon were less than 1.0, which is a key feature of non-Pb hyperaccumulator plant species (Fig. 5D), indicating that watermelon varieties are able to restrict much Pb in roots and greatly diminish upward movement to shoots, which might be a result of the binding of Pb with root cell wall ion exchange sites (Sun et al., 2020) and its extracellular precipitation in the form of Pb carbonates (Rucińska-Sobkowiak et al., 2013). Similarly, the strong binding ability of Pb with galacturonic and glucuronic acids in cell walls, and the restricting effect of the casparian strip are also responsible for its limited translocation (Wang et al., 2014).

Under Pb stress, MDA content increases in plant cells as a result of lipid peroxidation (Maodzeka et al., 2017). In our study, Pb stress induced more MDA generation in Pb-sensitive varieties (Fig. 6A and B). Comparable results have also been found in bamboo (Jiang et al., 2019), tobacco (Maodzeka et al., 2017), and *Coronopus didymus* (Sidhu et al., 2016). The increased MDA content in plants is a result of the formation of lipid peroxides from fatty acids. The greater MDA content is indicative of severe lipid peroxidation, which alters various physiobiochemical parameters as a result of oxidative stress (Sidhu et al., 2016).

Soluble protein and sugars (osmolytes) play important roles in plant defense against various abiotic stresses (Abdelkrim et al.,

Fig. 5. Pb accumulation and translocation of six watermelon varieties exposed to 20 μM Pb for 14 d. (A) Pb concentration in root. (B) Pb concentration in shoot. (C) Pb concentration in leaves. (D) Translocation factor of six watermelon varieties. Values given are mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($P \leq 0.05$) among varieties. DW, dry weight.

Fig. 6. Effect of 20 μ M Pb on malondialdehyde (MDA), total soluble protein (TSP), and total soluble sugar (TSS) in the leaves and roots of four watermelon varieties. Vertical bars represent the mean $\pm$ SD (n = 3). Values with different letters are significantly different ($P \leq 0.05$) among varieties. DW, dry weight; FW, fresh weight.

2018; Jiang et al., 2019). The decrease in soluble sugars and protein pools under Pb stress may cause severe oxidative stress in plants (Abdelkrim et al., 2018; Jayasri and Suthindhiran, 2017; Piotrowska et al., 2009). The synthesis and accumulation of soluble protein and sugars in plants under heavy-metal stress is known to play various important roles, such as protection and stabilization of cell membranes and enzymes, maintenance of turgor, and scavenging of reactive oxygen species (ROS)

(Abdelkrim et al., 2018; Ali et al., 2014; Ashraf et al., 2017; Rodriguez et al., 2012). The accumulation of soluble protein in leaves was significantly induced in Pb-tolerant varieties, but not in Pb-sensitive watermelon (Fig. 6C and D). The synthesis of soluble sugars was stimulated in both leaves and roots of all varieties (Fig. 6E and F), implying that the enhanced amount of soluble protein and sugars may be a possible tolerance strategy against Pb stress in watermelon varieties.

In our study, Pb reversely affected chlorophyll and carotenoid content in watermelon. Interestingly, the opposite response pattern of each pigment was observed between two tolerant varieties as well as two sensitive genotypes (Fig. 7). A correlation between chlorophyll variation pattern and translocation factor was observed in all watermelon (Figs. 5 and 7). A reduction in chlorophyll and carotenoid contents as a consequence of Pb toxicity has been observed in tomato (Bali et al., 2019), spinach (Natasha et al., 2020),

Fig. 7. Effect of 20 μM Pb on chlorophyll a, chlorophyll b, and carotenoids in the leaves of four watermelon varieties. Vertical bars represent the mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($P \leq 0.05$) among varieties.

tobacco (Maodzeka et al., 2017), *Iris pseudacorus* (Zhou et al., 2009), *Brassica napus* (Ferreiroa et al., 2017), and fodder turnip (Cenkci et al., 2010). Pb toxicity reduces chlorophyll content either by inhibiting its synthesis or enhancing degradation (Ferreiroa et al., 2017; Shakoor et al., 2014; Tian et al.,

2014). The inhibition of magnesium uptake and the decrease in the transport rate of iron to leaves by Pb may contribute to the high degradation rates of chlorophyll (Khan et al., 2016; Kumar and Prasad, 2018). Some studies have revealed the stimulatory effect of low doses of Pb on chlorophyll and carotenoid

contents in *Pogonatherum crinitum* (Hou et al., 2018), *Jatropha curcas* L. cuttings (Saikachout et al., 2015), *Atriplex* varieties (Shu et al., 2012), *Brassica napus* (Ferreiroa et al., 2017), and *Pseudochlorella pringsheimii* (Ismaiel and Said, 2018).

Under Pb stress, ROS are produced in plant tissues, which cause oxidative stress. To lessen this stress, plants stimulate their antioxidative defense system to scavenge the ROS (Ashraf et al., 2017; Venkatachalam et al., 2017). SOD is a metalloenzyme involved in detoxifying the superoxide radical to H_2O_2 and dimer oxygen (O_2), and thus eliminates the ROS. POD also detoxifies free radicals that usually cause lipid peroxidation of membranes, thus protecting cell membranes from oxidative damage. CAT plays a key role in scavenging H_2O_2 in peroxisomes, and thus diminishes oxidative stress (Jayasri and Suthindhiran, 2017; Shakoor et al., 2014). APX, another enzyme present in chloroplasts, catalyzes the breakdown of H_2O_2 into water and O_2 (Sidhu et al., 2016). In our study, significant and stronger enhancement of SOD, POD, CAT, and APX activities were observed in Pb-tolerant (V4 and V7) watermelon varieties, rather than in the more sensitive varieties (V3 and V8; Fig. 8), suggesting that the antioxidant system may contribute to Pb resistance in watermelon. Comparable results have also been reported in fragrant rice cultivars (Ashraf et al., 2017), tobacco (Maodzeka et al., 2017), and *Peganum harmala* (Mahdavian et al., 2016), in which the tolerant plants had elevated antioxidant enzyme activity compared with the sensitive plants. All these findings clearly suggest that the tolerant plants are better equipped to cope with Pb stress because of their greater antioxidant defense capability, in contrast to sensitive varieties.

Plant roots are the first response organ because of their direct contact with Pb in the environment. As a result of Pb toxicity, a sharp decrease in root growth occurs that might be the result of the inhibition of cell division in the root tip cells (Kumar and Prasad, 2018; Siddiqui, 2012). In a recent rice screening study, the only morphological difference between the Pb-tolerant and -sensitive rice varieties and cultivars was found in the growth of roots (Yang et al., 2000). Indeed, many studies have reported that inhibition of root elongation should be one of the most prominent effects induced under Pb and other heavy-metal stress (Hattab et al., 2016; Wang et al., 2011). To see whether root length could be used as an indicator parameter for determining Pb tolerance and sensitivity among watermelon varieties, we performed PCA based on all relative growth parameters. According to our analysis, the relative growth parameters were graded in PC1 and PC2 based on their loaded eigenvalues from highest to lowest. The analysis identified the RRL, RSFW, and RSDW (RRL = 0.937, RSDW = 0.896, and RSFW = 0.792 eigenvalues, respectively) as the major growth parameters, which contributed to the maximum variation in principal components

Fig. 8. Effect of 20 μ M Pb on superoxide (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) in the leaves and roots of four watermelon varieties. Vertical bars represent the mean $\pm$ SD ($n = 3$). Values with different letters are significantly different ($P \leq 0.05$) among varieties. FW, fresh weight.

(Fig. 9A and B). Furthermore, RRL correlated significantly with RSW and RSDW in both Pb-tolerant and -sensitive watermelon varieties. Taken together, these results suggest that RRL is a valid indicator parameter for the assessment of Pb tolerance and sensitivity at the seedling stage,

predicting good development and high biomass production of the aboveground parts of watermelon. Similar results were reported in *Medicago truncatula*, in which relative root growth was used as an indicator parameter for the assessment of mercury tolerance at the seedling stage, and

was confirmed, as plants with the greatest tolerance to this parameter showed good development of aerial parts (de la Torre et al., 2013). This could also be the main reason that root length is usually used as a measure for determining heavy-metal tolerance in plants.

Fig. 9. The component plot in the rotated space of principal components 1 and 2 obtained from principal component analysis-determined Pb tolerance indicator parameters based on their relative growth parameters according to their Eigen values. RLA, relative leaf area; RRDW, relative root dry weight; RRFW, relative root fresh weight; RRG, relative root growth; RSDW, relative shoot dry weight; RSFW, relative shoot fresh weight; RSL, relative shoot length.

Conclusions

Our study concluded that 1) 2 weeks of exposure to 20 μM Pb (optimum condition) caused significant nonlethal toxicity in watermelon seedlings; 2) MFA identified and classified the most Pb-tolerant (V4 and V7) and the most Pb-sensitive varieties (V3 and V8); 3) the tolerant varieties exhibited a differential Pb tolerance mechanism resulting from the significant difference in the translocation of Pb; 4) in contrast to Pb-sensitive varieties, Pb-tolerant varieties maintained high antioxidant activity and low MDA content, as well as a greater level of TSP content in leaves; and 5) PCA identified RRL as an indicator of Pb tolerance in watermelon.

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Supplemental Table 1. The composition of the Hoagland nutrient solution (full/half strength and modified).

S. no.	Chemical name	Hoagland's nutrient solution (chemical concn, mg/L)		
		Full strength	Half strength	Modified
1	KNO ₃	590	295	295
2	Ca(NO ₃) ₂ · 4H ₂ O	253	126.5	126.5
3	KH ₂ PO ₄	68	34	0.003
4	MgSO ₄ · 7H ₂ O	347	173.5	173.5
5	Na ₂ Fe-EDTA	30	15	15
6	H ₃ BO ₃	1.43	0.715	0.715
7	MnSO ₄ · H ₂ O	1.065	0.5325	0.5325
8	CuSO ₄ · 5H ₂ O	0.04	0.02	0.02
9	ZnSO ₄ · 7H ₂ O	0.11	0.055	0.055
10	(NH ₄) ₆ Mo ₇ O ₂₄ · 4H ₂ O	0.01	0.005	0.005