THE INFLUENCE OF SALICYLIC ACID FOLIAR SPRAY ON THE GROWTH, BIOCHEMICAL TRAITS, AND Cd-UPTAKE IN RADISH (RAPHANUS SATIVUS L.)

SYED HAMMAD RAZA1, FAHAD SHAFIQ2* AND SUMERA ANWAR2

1Department of Botany, Government College University Faisalabad, Pakistan
2Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan
*Corresponding author’s e-mail: fahadsheikh1800@gmail.com

Abstract

Due to agricultural mismanagement, cadmium (Cd) enrichment in cultivated soils raises concern over crop growth, productivity, and edible safety. In this present field study, the influence of salicylic acid (SA) on some growth, biochemical, and ion attributes of locally cultivated radish (Raphanus sativus L.) variety Mino towards Cd exposure is reported. The Cd-stress (0, 0.5, and 1 mM CdCl2) was applied to 20-d old plants. The foliar application of salicylic acid (0 and 300 mg L−1) was performed after 10-days of Cd exposure, and data for various growth and biochemical traits were recorded after 15-days of spray treatments. The inhibition of radish root growth was recorded even at 0.5 mM CdCl2 and severe inhibition was evident at 1 mM CdCl2 stress. In contrast, an increase in the shoot growth characters was noticed in response to Cd and SA treatments. Furthermore, Cd-stress reduced the chlorophyll contents, and these were further reduced by SA spray. Moreover, the SA-treated plants exhibited higher Cd contents in the shoot leading to chlorosis. The shoot Cd contents ranged between 6-57 µg/g DW while, the Cd concentrations in root were below 6 µg/g DW. Based on the results, we conclude that radish concentrated a majority of Cd-fraction in shoot compared to the root with minimal values.

Key words: Cadmium; Photosynthetic pigments; Salicylic acid; Metal toxicity; Vegetables.

Introduction

Cadmium (Cd) is a persistent environmental pollutant discharged extensively into agricultural lands from anthropogenic sources (Hegedűs et al., 2001; Han et al., 2015). Some of the prime sources of Cd release include the use of Cd containing inorganic phosphate fertilizers, sewage sludge application, and metal-working industries (Raza et al., 2014; Anwar et al., 2016). The relatively high mobility of Cd metal in the rhizosphere promotes rapid uptake by roots leading to its accumulation in different plant parts (Das et al., 1998). Consuming contaminated food exposes people to a lot of Cd, which can disrupt the normal body functions, resulting in short- or long-term health problems (Balali-Mood et al., 2021). For Instance, Cd attaches to albumin in plasma in the same way as zinc does, replaces calcium from bones and causes osteoporosis (Brzózska et al., 2001). In the same way it disrupts iron absorption causing anemia (Fujiwara et al., 2020) and altering zinc homeostasis (Urani et al., 2015). In addition, Cd accumulates in the kidney and liver, causing chronic nephrotoxicity and nephrocarcinogenicity (Lech & Sadlik, 2017), and designated as carcinogenic (group I) to humans by the International Agency for Research on Cancer (Anon., 1993).

When plants are exposed to Cd stress, they respond primarily by modulating biochemical and physiological processes. Once up-taken, Cd suppresses uptake of iron, zinc, and other nutrients (Xu et al., 2017), inhibits plant growth and carbon fixation (Drazkiewicz et al., 2003; Song et al., 2019), induces leaf chlorosis by degrading chlorophyll (Rydzynski et al., 2019), and disturbs photosynthetic apparatus (Perfus-Barbeeoch et al., 2002).

Because food is the main source of Cd toxicity, there is increased concern about Cd pollution in food and associated health consequences. Vegetables are one of the largest food sources causing Cd toxicity because they are usually cultivated in sub-urban areas with wastewater (Muchuweti et al., 2006; Hussain et al., 2013; Anwar et al., 2016). Many researchers studied Cd uptake and translocation in different vegetables under various soil conditions, which helps understand the uptake and transport mechanism. The concentration of Cd taken up by plants varies with the consumable part, vegetable type, and stage of vegetable growth (Shentu et al., 2008; Lai & Chen, 2013; Huang et al., 2021). In this context, Shentu et al., (2008) reported that radish accumulated more Cd in shoot than root compared to pakchoi and tomato.

Radish is a Brassicaceae vegetable with an edible root portion and is regarded as a model (indicator) crop for studying plant responses against various environmental pollutants, including heavy metals (Verma et al., 2007). However, its edible portion is directly in contact with the contaminated soil, which raises concerns over its edible safety and quality after harvest. On the other hand, salicylic acid (SA) is an important plant hormone and a signaling molecule (Guo et al., 2009). It has a crucial role in abiotic stress tolerance (Alvarez, 2000), including defensive mechanisms at low temperature, salt, and water stress conditions (Borsani et al., 2001). Exogenous application of SA on various species either through rooting medium or as foliar spray showed promising results in mitigating Cd toxicity in several plant species, including crops (Xu et al., 2015; Han et al., 2015; Sheng et al., 2015; Zhang et al., 2015; Shakerova et al., 2016), however, most often the studies are limited to laboratory conditions and their practical applicability is limited. Therefore, a practical approach was opted to investigate the influence of exogenously applied SA on Cd contents in radish plants under artificially Cd contaminated soils.

DOI: http://dx.doi.org/10.30848/PJB2022-5(28)

Materials and Methods

Experiment details: A field experiment was arranged in a split-plot design. Three main plots (4 m x 2 m) were prepared as described by Raza and Shafiq (2013a). Seeds of radish (Raphanus sativus L. variety Minio) were sown on both sides of the ridges. Three ridges were made in each main plot, the distance between ridges was 50 cm, and the height of each ridge was 15 cm. After 15 d of seed germination, plant to plant distance of 10 cm was maintained, and three levels of Cd, i.e., 0, 0.5, and 1 mM CdCl₂ were applied to the main plots. Then after 10 d of Cd exposure, plants on the first and second ridges (two rows each) were sprayed with salicylic acid (SA, 300 mg L⁻¹). 0.1% tween-20 was added as a surfactant and water sprayed, respectively, while plants on the third ridge were not sprayed (control).

Determination of growth and biochemical traits: After 15 d of SA treatments, plants were harvested, and various shoot and root lengths were measured. The shoot and root were weighed (fresh weight), dried at 70 °C, and weighed again (dry weight). Chlorophyll and carotenoid contents from the acetone (80%)-extracted leaf tissues were determined as described by Arnon (1949) and Kirk & Allen (1965).

Determination of inorganic ions: Finely powdered, dried shoot and root (0.5 g) samples were used for element analysis after wet-acid digestion (Netondo et al., 2002). The Cd content from the digested samples was determined by atomic absorption spectrophotometer (novAA400, Analytik Jena, Germany). Sodium (Na⁺) and potassium (K⁺) ions were determined using a flame photometer (Jenway PFP, UK).

Statistical analysis

Analysis of variance of the data was carried out using CoStat statistical software. Differences between means of treatments were assessed by Duncan’s Multiple Range test (p<0.05) using MStat software.

Results

Radish vegetative growth in response to Cd stress and salicylic acid foliar application: The exposure of radish to cadmium (Cd) contamination resulted in a significant reduction in root length (p<0.05), fresh root weight (p<0.001), and root dry weight (p<0.01) (Table 1). The application of salicylic acid (SA) did not improve the root length of radish plants compared to control plants, although improved root fresh and dry biomass in comparison with Cd-stressed (no spray) radish plants (p<0.05). In contrast, radish plants exposed to Cd contamination exhibited significantly higher shoot length (p<0.01) and fresh and dry biomass (p<0.01) than the control. A stimulation in the leaf area of Cd-stressed radish plants (p<0.001) and leaf numbers (p<0.05) were recorded. The foliar application of SA further improved the shoot growth attributes like shoot length; shoot fresh and dry weight, respectively (p<0.05). Furthermore, SA application increased the number of leaves and leaf area per plant (p<0.001) under Cd contamination.
Radish chlorophyll contents in response to Cd stress and salicylic acid foliar application: Exposure to Cd markedly reduced chlorophyll a (Chl a), chlorophyll b (Chl b), and total Chl contents in radish plants (Table 2). The maximum values were recorded in control, while the least was evident in plants exposed to 1 mM CdCl₂ contamination. The foliar application of salicylic acid improved the Chl a and b contents of control plants; however, its effect on these attributes of Cd-stressed plants was negative. We recorded significantly lower Chl a and b contents in response to SA foliar application ($p$<0.01). Therefore, SA application to Cd-stressed plants further reduced the total Chl contents of radish leaves, although it improved Chl contents of plants grown without cadmium.

Inorganic ions in radish plant parts in response to Cd stress and salicylic acid foliar application: The addition of CdCl₂ to the growth medium significantly enhanced plant Cd contents (Table 3). In the root, the Cd content ranged between 0.33 - 5.99 μg g⁻¹ DW ($p$<0.05), while in the shoot, it ranged 6.67 - 40.2 μg g⁻¹ DW ($p$<0.001) respectively. The foliar application of SA increased the root Cd contents that ranged between 0.31 - 4.90 μg g⁻¹ DW ($p$<0.05). Similarly, radish plants treated with SA exhibited shoot Cd concentrations that ranged between 11.2-57.4 μg g⁻¹ DW respectively ($P$<0.001). The addition of Cd to the growth medium and the foliar SA application differentially altered the Na⁺ contents of radish root and shoot. However, in contrast to Na⁺ ions, we recorded prominent uptake of K⁺ by radish plants in both root and shoot, respectively ($p$<0.001). The application of SA as foliar spray improved the root K⁺ contents ($p$<0.01); however, its effect on shoot K⁺ contents was insignificant.

Correlation of Cd contents in shoot and root with different growth indices: The Cd in shoot was positively related with Cd content in root, root length, and chlorophyll content (Fig. 1). The Cd fraction in root was negatively related with the fresh and dry weight of root and chlorophyll content. Leaf area was negatively related with ChlH and total Chl. Carotenoid content was not related with Cd content or any growth trait.

Discussion

Cadmium (Cd) is a hazardous, non-essential element with potential toxicity and carcinogenicity. It is one of the most dangerous elements due to its mobility, tendency to accumulate in the vegetative plant’s parts, and relatively less damaging effect on plants than on humans and animals. Vegetables can accumulate high levels of Cd and develop well with no visible phytotoxicity symptoms or yield decline (Wang, 2002; Liu et al., 2003; Shentu et al., 2008).

In this study, no decrease in the growth of radish aboveground plant parts was recorded even at the highest Cd application (1 mM of CdCl₂). The fresh weight of radish shoot showed 2.4 and 2.6- and 12.8-fold increase and dry weight were 1.85- and 2.24-fold increased at 0.5 and 1 mM of Cd, respectively than the control. Previous reports showed that Cd had no inhibitory effect on the shoot growth of radish (Shentu et al., 2008) or even had stimulated the growth of aboveground parts (Wu & Zhang, 2002; Ivanov et al., 2003; Liu et al., 2003; John et al., 2008; Januškaitienė, 2010) as found in the present study. The initial increase in growth by exposure to toxic ions might be due to hormesis (Poschenrieder et al., 2013), a response commonly evident in plants exposed to low concentrations of toxic ions (Calabrese, 2009).

Plants appear to be able to defend and protect their integrity against low metal stress. The low dose of Cd may serve as an activator of hormones and enzymes in cytokinin metabolism. Bruno et al. (2017) observed an increase in auxin level and enhancement of cytokinin signaling in Arabidopsis roots at lower Cd concentration (10 μM). Furthermore, plants cope with Cd stress by manufacturing low molecular weight Cd-binding proteins in response to low heavy metal stress, and this type of protein has been proven to play a role in metal tolerance (Epstein & Bloom, 2005).

In contrast to the aboveground parts, a dose-dependent reduction in the root growth attributes of radish plants was observed. The exposure to 0.5 mM and 1 mM Cd reduced the root fresh weight by 31 and 47% and root dry weight by 46 and 59%, respectively, as compared to control. Root is the first part of plants exposed to metal stress. The reduction in root growth can be attributed to Cd-induced morpho-anatomical modifications (Lux et al., 2011). Due to its small radius, Cd could replace Ca by entering cells through a Ca channel, binding to calmodulin, and interrupting cell division in root cells (Liu et al., 2003). SA application under Cd stress increased the root fresh weight by 30 and 75% and root dry weight by 38 and 94% compared to 0.5 mM and 1 mM Cd alone, respectively. These results showed the anti-stress, and beneficial effect of SA as SA enhances the membrane integrity, reduces the oxidative damage produced by Cd, lowers the malondialdehyde content, and electrolyte leakage (Belkhadi et al., 2010; Ahmad et al., 2011).

The application of salicylic acid (SA) increased the shoot fresh weight and dry weight by 75% and 100% under 0.5 mM Cd. Salicylic acid application during early growth stages helps plants avoid damage from Cd toxicity (Ahmad et al., 2011; Raza & Shafiq, 2013a) by expressing defense-related enzymes and particular proteins that help plants adjust themselves to reduce Cd toxicity (Chen et al., 2007; Gondor et al., 2016). Although under high Cd stress (1 mM CdCl₂), SA foliar reduced the shoot fresh and dry weight by 7% and 2% at 1 Cd stress. The decreased shoot biomass at high Cd might be due to the high Cd accumulation in the aboveground by SA spray.

Furthermore, an increase in the leaf area might be an adaptive response to compensate for a reduced photosynthetic rate. In this context, increased investment in the leaf is linked to carbon gain via better photosynthetic efficiency and faster growth rates to complete the life cycle rapidly (Poorter & Remkes, 1990). We integrate Cd-induced increased foliage growth with hormesis, which urges further exploration at the molecular level.
Table 2. Photosynthetic pigments of radish under varying cadmium chloride regimes in response to exogenous salicylic acid application.

<table>
<thead>
<tr>
<th>Cadmium (mM CdCl₂)</th>
<th>Foliar treatment</th>
<th>Chlorophyll a (mg g⁻¹ FW)</th>
<th>Chlorophyll b (mg g⁻¹ FW)</th>
<th>Total chlorophyll (mg g⁻¹ FW)</th>
<th>Carotenoids (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No spray</td>
<td>2.73b ± 0.08</td>
<td>0.843e ± 0.10</td>
<td>3.58e ± 0.19</td>
<td>0.338bd ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2.78b ± 0.21</td>
<td>0.870f ± 0.09</td>
<td>3.65f ± 0.31</td>
<td>0.347bc ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>3.18c ± 0.22</td>
<td>0.950g ± 0.10</td>
<td>4.13g ± 0.32</td>
<td>0.438g ± 0.05</td>
</tr>
<tr>
<td>0.5</td>
<td>No spray</td>
<td>2.41b ± 0.17</td>
<td>0.653d ± 0.01</td>
<td>3.06d ± 0.17</td>
<td>0.389d ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2.44b ± 0.19</td>
<td>0.731f ± 0.04</td>
<td>3.17f ± 0.15</td>
<td>0.410f ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>1.74c ± 0.09</td>
<td>0.509d ± 0.02</td>
<td>2.25d ± 0.12</td>
<td>0.287d ± 0.03</td>
</tr>
<tr>
<td>1</td>
<td>No spray</td>
<td>1.89c ± 0.23</td>
<td>0.560f ± 0.03</td>
<td>2.45f ± 0.26</td>
<td>0.345f ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>1.91c ± 0.17</td>
<td>0.583f ± 0.04</td>
<td>2.50f ± 0.21</td>
<td>0.350f ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>1.32d ± 0.18</td>
<td>0.476e ± 0.04</td>
<td>1.80d ± 0.22</td>
<td>0.242d ± 0.02</td>
</tr>
</tbody>
</table>

Salicylic acid was applied at 300 mg L⁻¹; Different letters on mean values (mean ± SE; n=3) in each column indicate significant difference

Table 3. Concentration of sodium, potassium, and cadmium content in radish under varying cadmium chloride regimes in response to exogenous salicylic acid treatment.

<table>
<thead>
<tr>
<th>Cadmium (mM CdCl₂)</th>
<th>Foliar treatment</th>
<th>Sodium (mg g⁻¹ DW)</th>
<th>Potassium (mg g⁻¹ DW)</th>
<th>Cadmium (μg g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>0</td>
<td>No spray</td>
<td>23.5b ± 3.0</td>
<td>33.9b ± 2.7</td>
<td>20.8b ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>24.7b ± 3.2</td>
<td>35.6b ± 2.9</td>
<td>21.9b ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>31.0c ± 1.4</td>
<td>43.7c ± 4.4</td>
<td>41.5c ± 5.0</td>
</tr>
<tr>
<td>0.5</td>
<td>No spray</td>
<td>22.1b ± 2.1</td>
<td>21.9d ± 3.0</td>
<td>47.0d ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>23.3b ± 2.3</td>
<td>23.1d ± 3.1</td>
<td>49.5d ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>23.9b ± 1.4</td>
<td>25.1d ± 2.3</td>
<td>50.0c ± 3.3</td>
</tr>
<tr>
<td>1</td>
<td>No spray</td>
<td>32.5a ± 2.8</td>
<td>35.4b ± 1.8</td>
<td>44.5e ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>34.2a ± 2.9</td>
<td>37.3b ± 1.9</td>
<td>46.8a ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Salicylic acid</td>
<td>31.0a ± 2.9</td>
<td>31.8bc ± 2.4</td>
<td>46.2a ± 6.9</td>
</tr>
</tbody>
</table>

Salicylic acid was applied at 300 mg L⁻¹; Different letters on mean values (mean ± SE; n=3) in each column indicate significant difference
We also recorded a reduction in chlorophyll contents in a Cd dose-dependent manner with maximum values for control plants. Despite the decrease in Chl a, Chl b, total chlorophyll with an increase in CdCl₂ doses, more pigment reduction was evident in salicylic acid (SA) treated plants under Cd stress, and this is consistent with the possibility discussed above. No positive effects of SA application can be linked with osmotic water correlations (Larque-Saavedra, 1978). Still, in the present study, these adverse effects were prominently evident in CdCl₂ harmful levels that can be explained further. Salicylic acid is reported to improve nutrient uptake (Glass, 1974). We propose that SA application might increase the uptake of Cd, and high Cd contents in SA-treated plants were confirmed through element analysis of shoots.

The Cd stress is known to decrease photosynthetic pigments, especially the Chl reduction is more apparent (Pandey, 2000). Similar reports were documented by Abdel-Latif et al. (2008), Pandey (2000), and Farouk et al. (2011). Lanaras et al. (1993) attributed this Cd-mediated Chl reduction to inhibition of certain enzymes like ALAdehydratase and Protochlorophyll reductase involved in Chl biosynthesis. In addition, some authors linked this reduction in pigments with the impaired uptake of iron and magnesium (Anwar et al., 2017). Alternatively, substituting magnesium in the Chl molecules by Cd is also responsible for this (Kupper et al., 1998). In addition, Anuradha et al. (2009) attributed these reductions in pigments to imbalanced water relations and damage to chloroplast structure.

Above all, in terms of Na⁺ ions, a differential response was evident, while on the other hand, Cd enrichment increased the uptake of K⁺ ions in both the root and shoot of radish plants. Contrary to these findings, a reduction in the K⁺ contents of sugarcane calli exposed to 1 mM CdCl₂ is reported (Raza et al., 2013b). Generally, Cd enters through K⁺ and Ca²⁺ channels and impairs these channels, which disturb ion homeostasis (Li et al., 2012). However, we recorded increased K⁺ contents, and SA application further enhanced these ions in radish plant parts.

The addition of CdCl₂ to the growth medium significantly enhanced Cd contents in radish plant parts. The storage roots of radish accumulated a much lower concentration of Cd (0.33 - 5.99 μg g⁻¹DW) than in the aboveground shoot part (6.67 - 40.2 μg g⁻¹ DM). Similar results were also reported by Shentu et al. (2008). Vegetables easily uptake and translocate heavy metals from the soil to the shoots via transpiration-based passive uptake (Marchiol et al., 2004). Therefore, the amount of Cd in the edible part of the vegetable is positively associated with the amount of Cd in the soil, represented by a linear or quadratic equation (Shentu et al., 2008). Cd is very mobile, and therefore it enters plants from the roots and is then transported through the symplastic pathway in the xylem tissue as a result of sap and/or transporters through shoots (Dong et al., 2019) or enters into the xylem through the apoplastic pathway (Kuriakose & Prasad, 2008).

The foliar application of SA increased shoot Cd contents that ranged between 11.2-57.4 μg g⁻¹ DW, respectively. At 1 mM of Cd, the foliar spray of SA increased the Cd content in shoot by 43% as compared to no spray, while the root Cd was unaffected. Similar results were also reported by Gondor et al. (2016). The high uptake of Cd by shoot might be due to the high synthesis of phytochelatins in the roots and their sequestration with Cd and transportation to the shoots (Gondor et al., 2016). These results indicate that radish has a high tolerance and accumulating potential of Cd and therefore accumulate a higher Cd concentration in the aboveground part compared to roots. Foliar application of SA improved the aboveground biomass and Cd uptake by shoot, but chlorophyll content, the biomass of storage root, and Cd uptake by root remained unaffected.

References


Larque-Saavedra, A. 1978. The antitranspirant effect of acetylsalicylic acid on *Phaseolus vulgaris* *L.* *Plant. Physiol.*, 43: 126-128.


Muchuweti, M., J.W. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J.N. Lester. 2006. Heavy metal content of...


(Received for publication 12 June 2021)