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COMPLEXATION OF CADMIUM WITH ORGANIC ACIDS IN XYLEM FLUID OF CHICORY AND PLANTAIN

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Abstract

Recent studies indicate that elevated levels of Cd in New Zealand agricultural soils can lead to high Cd accumulation in forage species such as chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.). These studies suggest the different abilities of pastoral species to either absorb Cd by roots or to translocate it from roots to shoots. Hence, it is important to determine the Cd translocation mechanism in these forage species. Plants produce Low Molecular Weight Organic acids (LMWOAs), which are involved in heavy metal translocation in plant xylem fluid. Therefore, a hydroponic experiment was conducted to evaluate the influence of increasing Cd concentrations on the production of LMWOAs in chicory and plantain xylem fluid. Germinated seedlings were separately grown in six different concentrations of Cd solutions (0, 0.01, 0.1, 0.5, 2.5 and 5 mg Cd/L) for 12 weeks and the LMWOAs concentrations in xylem fluid were analysed using High-Performance Liquid Chromatography. The results showed that oxalic, fumaric and citric acids in chicory, and oxalic and fumaric acids in plantain were the major LMWOAs in xylem fluids for all treatments. The fumaric and oxalic acid concentrations in chicory significantly increased ($p<0.05$) at 2.5 and 5 mg Cd/L, respectively. The respective percentage increases were 95% and 108% compared to control. The oxalic acid concentration in plantain nominally varied up to 0.1 mg Cd/L treatment and significantly ($p<0.05$) decreased by 26% at 0.5 mg Cd/L treatment relative to control. The citric acid concentration in chicory and fumaric acid concentration in plantain were independent of the increasing Cd levels in the solution. The fumaric acid concentration in chicory was significantly and positively correlated ($p<0.05$, $r= 0.99$) with the xylem fluid Cd concentration in chicory, while LMWOAs in plantain did not show any correlation with xylem fluid Cd concentration. In conclusion, it can be suggested that the translocation of Cd^{2+} in chicory can be facilitated by complexation with fumaric acid in xylem sap. However, further research is needed to confirm the findings of this study.

1.Introduction

In New Zealand, Cadmium (Cd) is a key environmental contaminant associated with the long-term high-rate application of superphosphate fertilizer, particularly on soils used for dairying and horticulture (Loganathan et al., 2003). Despite being a non-essential trace element, Cd can be absorbed by plant roots and transported to aerial parts (Senden and Wolterbeek, 1990). In 1990, new forage species such as chicory and plantain were introduced to the New Zealand modern livestock grazing systems, due to their high drought tolerance, nutrient content and environmental benefits. However, a recent study has revealed that the presence of elevated levels of Cd in New Zealand soils can result in increased Cd concentrations in forage plant species such as chicory and plantain relative to grasses and legumes (Stafford et al., 2016). Further, grazing on these Cd-rich forages will exceed the maximum guideline level for Cd in kidneys and livers (kidney 2.5 mg Cd/kg FW, liver: 1.25 mg Cd/kg FW (ANZFSC, 2012)) of grazing animals (Lee et al., 1994). Available literature suggests that pasture species have different abilities to absorb Cd from soils and translocate this element from root to shoot. Therefore, it is important to understand the mechanism related to Cd uptake by roots and translocation in xylem fluid/sap of chicory and plantain to avoid high Cd accumulation in kidneys and livers of livestock via continuous grazing in pastoral soils showing relatively high levels of Cd.

In general, heavy metal stress in the soil can activate various enzymes in the Tricarboxylic acid cycle (TCA cycle) of plants, which are responsible for the production of Low Molecular Weight Organic acids (LMWOAs) inside the plant cells (Tatár et al., 1998, Mnasri et al., 2015). Further, many studies have suggested that this high production of LMWOAs in various plant cells involve in root-to-shoot translocation of plants in the form of bound complexes (Lu et al., 2013). For example, Senden and Wolterbeek (1990) reported that metal–chelate complexes, such as Cd–citrate, in tomatoes could be transported Cd more efficiently from root to shoot via xylem sap. However, there have been no studies published to pinpoint the Cd uptake mechanisms of forage species, including chicory and plantain used in New Zealand agriculture. Therefore, a study was conducted to determine the effect of increasing Cd levels in hydroponic solution on the type and quantity of LMWOAs in xylem sap and the complexation of Cd with LMWOAs for Cd translocation in chicory and plantain xylem.

1. Materials and methods

2.1. Hydroponic experiment

The hydroponic experiment was set up in a greenhouse at the Massey University Plant Growth Unit with a day/night temperature of 17/20 °C. The composition of the basal nutrient solution was a Hoagland solution with six different Cd treatments 0 (control). 0.01, 0.1, 0.5, 2.5 and 5.0 mg Cd/L. Prior to the experiment chicory and plantain seeds were germinated on microfiber sponges for 10 days in a germination laboratory at 17/20 °C. The experiment was arranged in a completely randomized design with six treatments (6 containers (42L) per each plant) and five replicates per treatment.

2.2. Xylem fluid collection

Plants were grown for 12 weeks before harvest. At harvest, shoots were removed, and xylem sap was collected by the method described by Liao et al. (2000) with modifications. Briefly, the chicory and plantain stems were served using a stainless-steel razor blade at about 1cm above the media surface perpendicular to the stem axis and the xylem sap was collected with a micropipette. The saps were immediately frozen at -80 ˚C after collection until further analysis.

2.3. Total Cd concentration in xylem fluid

Xylem sap Cd concentration was determined based on the method explained by Nakamura and Akiyama (2008).

2.5. HPLC analysis for low molecular weight organic acids in xylem fluid

The composition and concentration of LMWOAs in xylem sap were analysed by High-Performance Liquid Chromatography (HPLC) (Agilent Technologies 1200 Series, Santa Clara, CA, USA) as described by Cawthray (2003) and Nakamura and Akiyama (2008). Briefly, 10 μL of the xylem exudate sample were diluted with 990 μL of HPLC mobile phase solution 25 mM KH_2PO_4. Identification of organic acids was performed by comparing retention times in root exudate samples with those retention times obtained by analysing of a standard mixture including six organic acids (i.e. acetic, citric, fumaric, malic, oxalic and tartaric acids).

2. Results and discussion

3.1. Composition and concentration of LMWOAs in root exudates

Oxalic, fumaric and citric acids in chicory, and oxalic and fumaric acids in plantain were the major LMWOAs in all Cd treatments. The concentration of citric acid in chicory xylem sap did not show any trend with the increasing Cd concentration of the hydroponic solution. Further, the fumaric acid concentration in chicory did not significantly differ ($p>0.05$) between the control, 0.01, 0.1 and 0.5 mg Cd/L treatments, but significantly ($p<0.05$) increased by 95% at the 2.5 mg Cd/L Cd treatment relative to the control. Similarly, the oxalic acid concentration in chicory did not significantly ($p>0.05$) differ up to 2.5 mg Cd/L treatment and significantly ($p<0.05$) increased by 108% at 5 mg Cd/L treatment relative to control (Figure 1). These results are in agreement with Li et al. (2019) who reported an increase of oxalic acid concentration by 55% in a rice variety when the Cd concentration of hydroponic media increased from control to 2 mg Cd/L and they have found a significantly positive correlation ($p<0.01$ $r = 0.90$) between Cd concentration and oxalic acid concentration in rice xylem sap. They have suggested that this observation may be due to the influence of high Cd level on the activity of several enzymes in the TCA cycle which promotes the synthesis of oxalic acid in rice (López-Millán et al., 2009). However, among all LMWOAs in chicory, only the fumaric acid significantly and positively correlated with the xylem sap Cd concentration (Table 1). It was suggested that fumaric acid can chelate metals strongly due to its dicarboxylic functionality (Xin et al., 2017) and importantly, it has high affinity and high stability constants towards Cd^{2+} ions compared to other LMWOAs ($pKa1=2.98$, $pKa2=4.34$) (Adeniji et al., 2010). These results suggest that fumaric acid may have an active involvement, via complexation, in Cd translocation towards areal parts of chicory via xylem sap.

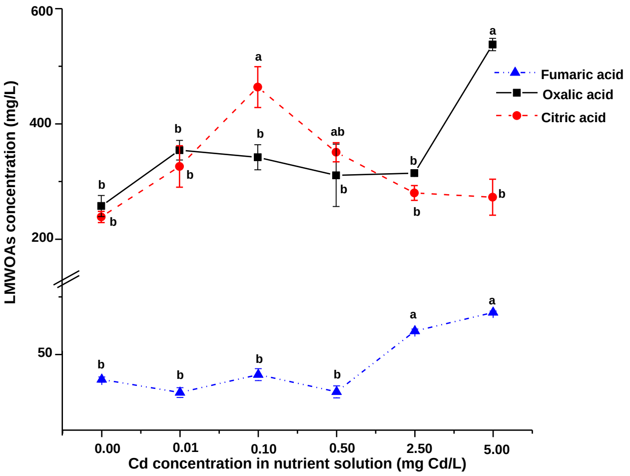

Figure 1 LMWOAs concentrations at different Cd treatments in chicory. Values in each point, at specific LMWOA followed by the different letter are significantly different at $p<0.05$ ($n=5$).

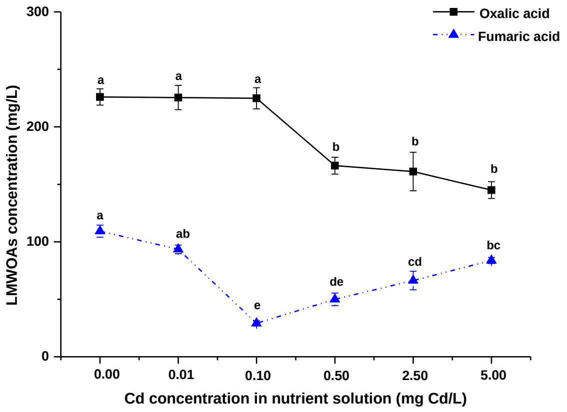

Figure 2 LMWOAs concentrations at different Cd treatments in plantain. Values in each point at specific LMWOA, followed by the different letter are significantly different at $p<0.05$ (n=5).

Table 1: Correlation analysis between Cd concentration (in xylem sap) and organic acids in xylem sap of chicory and plantain which were grown in hydroponic media containing increasing Cd concentrations.

Plant type	Oxalic acid	Fumaric acid	Citric acid
Chicory	0.66	0.99*	0.27
Plantain	-0.89*	-0.24	ND

Using Pearson correlation coefficient (r) to measure the relationship between variables. *Significant correlation level at $p<0.05$. ND= not detected.

The oxalic acid in plantain xylem sap did not significantly ($p>0.05$) vary between control, 0.01 and 0.1mg Cd/L treatments but significantly ($p<0.05$) decreased by 26% at 0.5 mg Cd/L treatment compared to control (Figure 2). In addition, the fumaric acid in plantain xylem sap did not show any trend with the increasing concentration of Cd in the hydroponic culture (Figure 2). Even though there was no significantly ($p>0.05$) positive correlation observed between the xylem sap Cd concentration and xylem sap LMWOAs in plantain, there was a significantly ($p<0.05$) negative correlation observed between xylem sap Cd concentration and oxalic acid concentration in plantain xylem sap. However, several studies have reported that plantain accumulates more Cd in roots without translocating it via xylem sap towards shoots (Abe et al., 2008; Crush et al., 2019). Therefore, these results suggest that in plantain LMWOAs may form complexes with Cd in roots which prevent Cd translocation via xylem towards the shoot. For example, Javed et al. (2017) found, in maize plants, that exudation of organic acids in response to Cd stress promoted accumulation of Cd in root tissues by bonding the metal in a non-toxic form that reduced the free Cd available, thus alleviating Cd toxicity. However, further research is needed to clarify if this detoxification mechanism is active in Plantain roots

4. Conclusion

The results of the current study showed that the translocation of Cd^{2+} in chicory can be mostly facilitated by the complexation with fumaric acid in xylem sap. Further, no LMWOAs Cd complexation was observed in xylem sap of plantain may be due to the high Cd accumulation in plantain roots than shoots. However, further research is needed to confirm the findings of this study.

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