

学位論文要旨 Dissertation Abstract

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学位論文題目 : Study on physiological and biochemical mechanisms of acetate-induced abiotic stress tolerance in lentil (*Lens culinaris* Medik)
Title of Dissertation (レンズ豆 (*Lens culinaris* Medik) における酢酸塩誘導-非生物的ストレス耐性の生理・生化学的メカニズムに関する研究)

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To meet the increased demand of food, we need to produce more food. However, environmental conditions such as soil salinity and heavy metal toxicity (copper, Cu; arsenic, As; cadmium, Cd) are threatening not only crop production but also human health. To sustain crop production under these stressful conditions, we used chemical biology approach where chemicals are used to induce tolerance in plants against different stresses by stimulating the inherent tolerance mechanisms in plants. Here, I conducted four experiments to evaluate the potential of a low cost and non-toxic chemical, acetate, in mitigating salt stress and heavy metal toxicities in lentil seedlings. In the first experiment, responses of lentil seedlings exposed to 100 mM salinity stress with or without 5 and 10 mM Na-acetate were investigated. Results showed that salt stress reduced recovery percentage, fresh weight (FW), chlorophyll (chl) content, disturbed water balance, disrupted antioxidant defense pathway by decreasing ascorbate (AsA) content, and caused ion toxicity resulted from increased Na^+ accumulation, severe K^+ loss from roots in hydroponic culture. However, exogenous application of acetate improved the seedling growth by maintaining water balance and increasing chl content. Furthermore, acetate application reduced oxidative damage by modulating antioxidant defense pathway, and sustained ion homeostasis by reducing Na^+ uptake and K^+ loss. In the glass house, we investigated the long-term effect of salinity with or without acetate on lentil seedlings. Acetate application increased FW and dry weight, reduced oxidative and membrane damage, and lowered the accumulation of Na^+ in shoot compared with salt stressed seedlings alone.

In the second study, we investigated the mechanisms underlying acetate-mediated Cu toxicity tolerance in lentil. Results demonstrated that high dose of Cu (3.0 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) reduced seedling growth and chl content, while augmenting Cu contents in both roots and shoots, and increasing oxidative damage in lentil plants through disruption of

the antioxidant defense. However, acetate pretreatment reduced Cu accumulation in roots and shoots, increased proline content and improved the responses of antioxidant defense (e.g. increased catalase and glutathione-*S*-transferase activities, and improved action of glutathione-ascorbate metabolic pathway). As a result, excess Cu-induced oxidative damage was reduced, and seedling growth was improved under Cu stress conditions, indicating the role of acetate in alleviating Cu toxicity in lentil seedlings. Taken together, exogenous acetate application reduced Cu accumulation in lentil roots and shoots and mitigated oxidative damage by activating the antioxidant defense, which were the major determinants for alleviating Cu toxicity in lentil seedlings.

In the third study, we investigated the mechanism of As tolerance in acetate-treated lentil seedlings. Results showed that both levels of As caused severe chlorosis, growth reduction and water imbalance. Furthermore, As-induced oxidative damage in plants manifested by higher malondialdehyde content, hydrogen peroxide content, and disruption of antioxidant defense pathway. However, acetate pretreatment improved growth and chl content, and reduced oxidative damage by enhancing some components of antioxidant defense pathway in seedlings. Under As stress condition, accumulation of As was found in both roots and shoots of lentil. However, acetate pretreatment reduced As accumulation in roots and inhibited the transfer of As to the shoots in lentil seedlings.

In the fourth study, we investigated the role of acetate in mitigating Cd stress tolerance. Results suggested that high dose of Cd negatively affected the shoot dry weight, root dry weights and photosynthetic pigments. Furthermore, Cd stress induced severe oxidative damage and accumulation of Cd in both roots and shoots. Interestingly, acetate pretreatment improved the growth parameters, photosynthetic pigments and phenotypic appearance of lentil seedlings under Cd stress indicating that acetate application enhances Cd stress tolerance in lentil. Further investigation revealed that acetate pretreatment increased catalase activity and AsA content, and reduced Cd translocation which in turn alleviated the oxidative damage in Cd stressed seedlings.

To conclude, from these series of experiments, it is suggested that acetate is a good candidate to induce multiple stress tolerance in lentil. Even though, under different stresses, responses of lentil seedlings varied with or without acetate, acetate treated seedlings showed some common responses such as enhancement of catalase activity and AsA content, and inhibition of transfer of toxic ion to the more sensitive aerial parts of lentil seedling which lead to better growth and phenotypic appearance.