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SUMMARY OF DOCTORAL THESIS

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Title: Nutrio-physiological study of salinity tolerance mechanism in rosemary (*Rosmarinus officinalis* L.)

（ローズマリー(*Rosmarinus officinalis* L.)における耐塩性機構に関する栄養生理学的研究）

Salinity in cultivated land is one of the main problems affecting food security because it reduces crop growth and productivity (Butcher et al., 2016). The stress imposed by salinity on the plant is due to the fact that the water is osmotically retained in the saline solution surrounding the roots, and becomes less accessible to the plant. Subsequently, an excessive absorption of salt could disturb the uptake of essential nutrients like K, Ca, or Mg, which harms biochemical and physiological processes in the plant. If the osmotic and ionic adverse effects of salt absorption exceed the tolerance level of the plant, oxidative stress occurs (Larcher, 2013). Rosemary is a Labiatae aromatic herb widely cultivated in arid and semi-arid regions because of its medical and cosmetic benefits and moderate salinity tolerance. Different characteristics of rosemary have already been evaluated under salinity conditions, however, were made in a single variety, therefore, the mechanism of tolerance underlying such characteristics have not been investigated. In this study, we aimed to elucidate the salinity tolerance mechanism in rosemary from the view point of selective absorption and transport of K over Na, and nutritional and physiological aspects comparing with other Labiatae aromatic herb species and using 8 rosemary varieties.

At first, the selective absorption and transport of K over Na, and nutritional and physiological characteristics were measured in rosemary plants (*Rosmarinus officinalis* L.) to elucidate the responses that can lead to acclimation or tolerance in this species. Sodium is the most abundant salt that competes with nutrients in the absorption by plants, mainly with K, an essential nutrient which is crucial for cell osmo-regulation, stomatal function, activator of large number of enzymes involved in photosynthesis, protein synthesis and oxidative metabolism. Therefore, we focused on the processes that regulate the salt content in the plant, the selective absorption and selective transport of K over Na in rosemary and thyme, two Labiatae herbs with similar salinity tolerance. The results revealed that in low-salinity (ECe 3 dS m^{-1}) rosemary and thyme doubled the selective absorption of K over Na by the roots ($SA_{K.Na}$) with respect to the control, but only thyme presented a remarkable increase in the selective transport of K over Na from the roots to the stems [$ST_{K.Na(roots/stems)}$]. Under high-salt conditions (ECe 6 dS m^{-1}), rosemary suppressed the $SA_{K.Na}$, that might inhibit the $ST_{K.Na\ (root/stem)}$ and $ST_{K.Na\ (stem/leaves)}$, leading to growth reduction. On the other hand, in thyme the increased $SA_{K.Na}$ could stabilize $ST_{K.Na\ (root/stem)}$ similar as in the control, but a decrease in $ST_{K.Na\ (stem/leaves)}$ may contribute to a decrease in dry matter due to an ionic imbalance.

At second, we aimed to evaluate the nutritional status of rosemary affected by salinity that could lead to functional damage and growth reduction. Eight rosemary varieties were hydroponically cultivated in moderate (50 mM NaCl) and high (100 mM NaCl)

salinity condition. Control was set as 3 mM NaCl. At 50 mM NaCl, the relative tolerance index (RTI), relative growth to non-saline condition, decreased in the order of Primley Blue >> Lockwood de Forest ≈ Prostratus ≈ Salem ≈ Benenden Blue > Arp ≈ Tuscan Blue > Officinalis. The Na/K ratio and occurrence percentage of leaf necrosis were significantly and negatively correlated with the RTI through eight varieties. Leaf tissue tolerance, indicated by a low occurrence percentage of leaf necrosis with high leaf Na concentration, differed slightly among varieties, but Salem, Arp, and Tuscan Blue showed the highest tissue tolerance, however it could not contribute to the salinity tolerance in this species. At both 50 and 100 mM NaCl, most varieties reduced leaf concentration of K, Ca and Mg, but leaf water content showed little effect of salinity. At 100 mM NaCl, the correlation between the RTI and the Na/K ratio or occurrence percentage leaf necrosis became inconsistent. Therefore, rosemary might tolerate to salinity by maintaining appropriate levels of nutrients with a low Na/K ratio in leaves prior to water status. Although the transport of some Na to a leaf might improve osmotic adjustment, the excessive transport of Na to leaves creates ionic stress that leads to reduced growth.

At third, physiological aspects, specifically characteristics of gas exchange and antioxidative response system, were investigated to understand the salinity tolerance mechanisms in rosemary. At 50 mM NaCl, most varieties reduced photosynthetic rate (A) and stomatal conductance (gs), and both parameters were positively correlated (r=0.531**), which might be possibly to prevent damage to photosystem, adjust the osmotic pressure and make the use of water more efficient. The intercellular CO_2 concentration (Ci) and electron transport rate (ETR) were maintained stable compared to 3 mM NaCl. In addition, A was correlated with the antioxidative activity of catalase (CAT) (r=0.386*) and ascorbate peroxidase (APX) (r=0.410*), but APX was positively correlated with occurrence percentage of necrosis (r=0.610**). This result might suggest that increase of antioxidative enzymes activities could maintain A but excessive reactive oxygen species (ROS) led to increased occurrence percentage of leaf necrosis. Also superoxide dismutase (SOD) activity increased in the most salt sensitive varieties, Tuscan Blue and Officinalis possibly for scavenging of the superoxide radical, suggesting that most tolerant varieties did not demand of increase of SOD activity. Total chlorophyll content was negatively correlated with occurrence percentage of leaf necrosis (r=-0.467**), gs (r=-0.365*), A (r=-0.377*) and activity of APX (r=-0.458**). This result suggests that reduction in the chlorophyll content could be a symptom of dehydration or related to oxidative stress induced by salt. Different physiological response to salinity stress was triggered in 100 mM. The glutathione reductase (GR) activity in all varieties was significantly correlated with several physiological parameters, although it was not affected by salinity except for Lockwood de Forest. Activity of GR was negatively correlated with RTI (r=-0.392*), A (r=-0.354*) and chlorophyll content (r=-0.440*), but positively with occurrence percentage of necrosis (r=0.516**).

Salinity tolerance mechanism in rosemary could be explained from view point of nutritional and physiological aspects. Selective absorption of K over Na from soil to root is markedly crucial to maintain proper leaf nutritional status. Rosemary absorbs K over Na and hold Na intensively in the root, although some Na absorbed plays a role as osmolyte in the leaf. Due to the significant negative correlation of RTI with Na/K ratio under conditions of moderate salinity, it is found that the Na/K could be an accurate indicator of salinity tolerance in this species. Antioxidative enzymes such as CAT and APX possibly scavenge effectively reactive oxygen species generated by salinity. ROS detoxifying mechanism might protect the photosystem from photoinhibition during changes of light under natural situations.