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SUMMARY OF DOCTORAL THESIS

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Title: Alleviation of Salt-Induced Injury of Plants by Scavenging Reactive Carbonyl Species
（活性カルボニル種の消去による，植物の塩ストレス障害の軽減）

Soil salinity is one of the most detrimental stresses that limits plant survival and hampers crop production. Among different abiotic stresses, soil salinity is a common problem. The soil which water-soluble salts exceed 4 dS m^{-1} (approximately 40 mM NaCl) is considered saline. Salinity is one of the major abiotic stresse that impairs crop productivity by causing ionic imbalance and osmotic stress, and as a result of metabolic distortion, the level of reactive oxygen species (ROS) is increased. ROS oxidize proteins, lipids and nucleic acids and eventually cause damage to cells and bring growth inhibition. The mechanism how ROS cause damage in cells have not been discovered yet.

We investigated the downstream product of the ROS, Reactive carbonyl species the downstream product of ROS which are the lipid peroxide-derived *α,β*-unsaturated aldehydes and ketones, in seed germination and plant growth retardation under salt stress. We took *Arabidopsis thaliana* Col-0 seeds and exposed them to 100 mM NaCl.

Then observed the germination percentages of seed. Germination of a seed was judged on its radicle emergence out of the seed coat. In the absence of NaCl, 90% seeds germinated at 24 h and nearly 100% at 36 h, while in 100 mM NaCl only 7% at 24 h and 38% at 36 h germinated. At 60 h, 100% seeds germinated regardless of salt treatment. Specifically, the 100 mM NaCl treatment caused mild stress, where germination was delayed but the seeds were not killed.

After germination, the seedlings were let grow by the 6th day and their dry weight was determined. The dry weight of the salt-stressed seedlings was lower by 55% than that of control seedlings Dry weight of carnosine-, Ac-Car- and anserine-treated salt stressed seedlings was only lower by 25%, 15% and 12% than unstressed seedlings, respectively. In the absence of NaCl, these dipeptides did not either increase or decrease the dry weight of seedlings.

Under the salt stress the levels of ROS, RCS, and protein carbonylation in the seedlings were increased. Acrolein, HNE, HHE, (*E*)-2-pentenal, (*E*)-2-hexenal), and four types of non-RCS aldehydes ((*Z*)-3-hexenal, *n*-heptanal, *n*-nonanal and *n*-decanal) were significantly increased in salt-stressed seedlings.

Adding the histidine-containing dipeptides carnosine, *N-acetylcarnosine* and anserine, which are reported RCS scavengers, restored the germination speed. The addition of dipeptides suppressed the increases in RCS and protein carbonylation but did not affect the ROS level.

We then tested the growth of seedlings in terms of dry weight. The six-day-old seedlings were transplanted to stress conditions, and the twelve-day-old plants were observed. The root growth was inhibited, formed leaves chlorosis and the dry weight of the seedlings was retarded. Addition of RCS scavenger dipeptide successfully mitigated these damages. Under salt stress RCS acrolein, HNE, HHE, crotonaldehyde, *E*-2-pentenal, *E*-2-hexenal were increased and addition of dipeptide suppressed the increased level these RCS. Thus, RCS are involved in inducing salt stress damages.

Then we investigated the roles of RCS produced from roots and shoots for the salt-induced damage of the plant of WT and the novel carbonyl scavenger enzyme 2-alkenyl

reductase (AER) overproducing transgenic lines COS-AER#3 and COS-AER#13. In this study, we used the six-day-old plants, transplanted to NaCl (90 mM) and at twelve-day-old seedlings, the growth was observed. The root growth was inhibited, formed leaves chlorosis and the plants' growth was retarded due to salt stress. To analyze the RCS accumulation in the shoot and root, we separated the root part from the shoot part. The salt treatment increased the contents of highly toxic RCS, *i.e*. acrolein, HNE and 4-hydroxy-2-hexenal (HHE), to twofold. These three RCS were also increased in the leaves, but their levels were lower than in the roots. The transgenic plants (COS-AER plants) in which the RCS-scavenging enzyme 2-alkenyl reductase (AER) is overexpressed under the β-estradiol (β-ED)-responsive promotor suffered much less damage under salt stress than did wild type when they were supplemented with β-ED. The addition of *β*-ED to COS-AER plants significantly restored the growth of plants under salt stress, while it did not have any effects on the growth of WT as judged on DW. The elongation of the primary root in COS-AER plants under salt stress also tended to recover in response to *β*-ED, while in WT, root elongation was not affected by *β*-ED. The addition of *β*-ED to the COS-AER lines suppressed the decrease in the Fv/Fm value due to salt stress, while in WT plants, it did not reduce the loss of PSII activity. Thus, the induced overexpression of AER in COS-AER lines improved the plant's tolerance against salt stress.

In shoots and roots several carbonyls, i.e. propionaldehyde, crotonaldehyde, HNE, (*E*)-2-hexenal, acrolein, HHE, propionaldehyde we detected. The contents of crotonaldehyde in leaves was 1.8 times lower than the contents found in roots. Similarly, the contents of HNE, (*E*)-2-hexenal, acrolein and HHE in leaves were lower than those in roots by 1.5 times, 8.0 times, 4.7 times and 6.6 times, respectively. Thus, shoots of *A. thaliana* contained fewer carbonyls of detectable levels than roots, and the basal levels of these carbonyls in leaves were lower than those in the roots. In the COS-AER plants, the basal level of crotonaldehyde was 1.3 times higher than WT. For other carbonyls, the basal levels were not significantly different between COS-AER and WT.

In salt stressed WT shoots, the contents of HNE, (*E*)-2-hexenal and HHE were higher than the non-stressed shoots by 2.0 times, 3.3 times and 1.6 times, respectively. The propionaldehyde level was also higher by 2.1 times. It should be noted that these RCS levels in shoots were lower even under the stress condition than their basal levels in roots. For example, the HNE level in stressed shoots was ca. 22 nmol (g FW)$^{-1}$, while that in non-stressed roots was ca. 28 nmol (g FW)$^{-1}$. For acrolein, 3.8 nmol (g FW)$^{-1}$ in stressed shoots and 11.5 nmol (g FW)$^{-1}$ in non-stressed roots. The crotonaldehyde content in shoots was not significantly changed by the salt stress treatment. In COS-AER plants with *β*-ED, the stress-induced increases in acrolein, HNE, and HHE were significantly suppressed in roots, but in leaves, HNE only was suppressed. Thus, the overexpressed AER primarily protected roots from RCS toxicity. Prevention of leaf chlorosis in COS-AER plants was probably a consequence of root protection and partially due to the HNE detoxification. These results demonstrate that accumulating RCS in roots under salt stress is a major cause of growth retardation.

Scavenging of RCS in plants either chemically of enzymatically could be an excellent tool for salt stress mitigation. Our findings reveal that scavenging RCS the downstream product of RCS playing a vital role to induce salt stress damages. The relevant RCS could be a future target ROS study.