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SUMMARY OF DOCTORAL THESIS

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Title: Studies of oxylipin carbonyl signals under oxidative stress in triggering programmed cell death in plants
（植物での酸化ストレスによるプログラム細胞死の引き金となるオキシリピンカルボニルの研究）

Reactive oxygen species (ROS)-triggered programmed cell death (PCD) is a typical plant response to biotic and abiotic stressors. Lipid peroxide-derived toxic carbonyl compounds (oxylipin carbonyls), products downstream of ROS, were recently revealed to mediate abiotic stress-induced damage of plants. We here investigated the biochemical mechanism by which oxylipin carbonyls triggers cell death in plant. When tobacco Bright Yellow-2 (BY-2) cells were exposed to H_2O_2, several species of short-chain oxylipin carbonyls, i.e., 4-hydroxy-(*E*)-2-nonenal and acrolein, accumulated and the cells underwent PCD as judged based on DNA fragmentation, an increase in TUNEL-positive nuclei and cytoplasm retraction. Oxylipin carbonyls caused PCD was also verified in the roots of tobacco and *Arabidopsis thaliana* after exposure with H_2O_2 and NaCl. The involvement of oxylipin carbonyls in the mediation of an oxidative signal to cause PCD, we performed pharmacological and genetic experiments. In pharmacological experiment we used carnosine and hydralazine, having distinct chemistry for scavenging carbonyls, significantly suppressed the increase in oxylipin carbonyls and blocked PCD in BY-2 cells and *A. thaliana* roots, but did not affect the levels of ROS and lipid peroxides. A transgenic tobacco line that overproduces 2-alkenal reductase, an *A. thaliana* enzyme to detoxify α,β-unsaturated carbonyls, suffered less PCD in root epidermis after H_2O_2- or NaCl treatment than did the wild type, whereas the ROS level increases due to the stress treatments were not different between the lines.

To investigate the biochemical action of oxylipin carbonyls in the cell death events we found that acrolein activated caspase-like proteases before appearing cell death morphology. We used two doses of acrolein namely lethal (0.2 mM caused PCD) and sub-lethal (0.1 mM did not cause PCD). These two doses of acrolein asserted critically different effects on the cells. Both lethal and sub-lethal doses of acrolein exhausted the cellular glutathione pool in 30 min, while lethal dose only caused a significant ascorbate decrease and ROS increase in 1-2 h. Prior to such redox changes, we found, acrolein caused significant increases in the activities of caspase-1-like protease (C1LP) and caspase-3-like protease (C3LP), the proteases to trigger PCD. Acrolein and 4-hydroxy-(*E*)-2-nonenal, another RCS, activated both proteases in cell-free extract from untreated cells. H_2O_2 at 1 mM added to the cells increased C1LP and C3LP activities and caused PCD, and the RCS scavenger carnosine suppressed their activation and PCD. However, H_2O_2 did not activate the proteases in cell-free extract. We also found that acrolein after 30 min exposure slightly but insignificantly up-regulated *VPE1a, VPE1b* genes, attribute to C1LP, although the up-regulation was significant after 1 h. Therefore, the activation of caspase-like proteases by RCS was the most critical and initial biochemical event in oxidative signal-simulated PCD in plants.

From these results we conclude that oxylipin carbonyls, downstream product of ROS, are involved in the PCD process in oxidatively stressed cells. We estimated the relative strengths of distinct carbonyl species and found that acrolein and 4-hydroxy-(*E*)-2-nonenal are the most potent carbonyls. Acrolein activated caspase-like proteases before changes the redox state of the cells. These results reveal the biochemical mechanisms of the oxylipin carbonyls-mediated initiation of PCD in plants. Our findings demonstrate a critical role of the lipid metabolites oxylipin carbonyls in ROS signaling.