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Relationship between chloroplastic H_2O_2 and the salicylic acid response

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Reactive oxygen species (ROS) act as signaling molecules for regulating plant responses to abiotic and biotic stress and there exist source- and kind-specific pathways for ROS signaling. Recently, we created a novel system for producing H_2O_2 in Arabidopsis chloroplasts by chemical-dependent thylakoid membrane-bound ascorbate peroxidase (tAPX) silencing using an estrogen-inducible RNAi method. Microarray analysis revealed that the expression of a large set of genes was altered in response to tAPX silencing, some of which are known to be involved in pathogen response/resistance. Furthermore, we found that tAPX silencing enhances the levels of salicylic acid (SA) and the response to SA, a central regulator for biotic stress response. In this addendum, we describe the relationship between chloroplastic H_2O_2 and SA in stress response, and discuss the function of the kind- and source-specific ROS signaling in SA-mediated stress response.

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Reactive oxygen species (ROS) act as signaling molecules involved in responses to abiotic and biotic stress in plants.[1-4] It has gradually been accepted that source- and kind-specific pathways exist for ROS signaling.[5] To understand the role of ROS in plant responses to stress, the molecular mechanism and signaling crosstalk of each pathway must be analyzed.

Chloroplasts are one of the most significant sources of ROS in pant cells. Thylakoid membrane-bound ascorbate peroxidase (tAPX) is a major H_2O_2-scavenging enzyme in chloroplasts.[6-8] To clarify the signaling function of chloroplastic H_2O_2, we recently created a novel system for producing H_2O_2 in Arabidopsis chloroplasts by estrogen-inducible silencing of thylakoid membrane-bound ascorbate peroxidase (tAPX), a major H_2O_2-scavenging enzyme in chloroplasts.[9] Microarray analysis revealed that tAPX silencing affects the expression of 774 genes. Functional classification of the chloroplastic H_2O_2-responsive genes and physiological analyses using the tAPX-silencing system indicated that chloroplastic H_2O_2 negatively regulates the response to chilling, and has antagonistic and synergistic roles in the response to high light. Furthermore, we found that tAPX silencing enhances the levels of salicylic acid (SA) and the response to SA, a central regulator for biotic stress response,[10,11] indicating crosstalk between chloroplastic H_2O_2 and SA in stress response.[9] In this addendum, we provide further data supporting the crosstalk, and discuss the function of source-specific H_2O_2 signaling pathways for regulating the SA response.

To study the effect of chloroplastic H_2O_2 on the SA response we checked the sensitivity of tAPX-silenced plants to SA treatment. As described in Maruta et al.[9] at 2 d after estrogen (100 μM) treatment, the expression of tAPX was drastically suppressed at the protein level in the IS-tAPX plants. IS-GUS plants were used as a control. Seventeen-day-old IS-GUS and IS-tAPX plants grown under continuous light at 100 μmol photons/m^2/s were treated with estrogen. At 2 d after the treatment, plants were further treated with a high concentration (5 mM) of SA for 4 d. As shown in **Figure 1**, the leaves of IS-GUS plants and estrogen-untreated IS-tAPX plants were visibly damaged by SA treatment to the same degree. Conversely, the leaves of estrogen-treated IS-tAPX plants were insensitive to the treatment, suggesting that chloroplastic H_2O_2 causes SA insensitivity. This result was unexpected, because our previous findings revealed that tAPX silencing enhances the levels of SA and the SA response. However, the SA-insensitive phenotype of the tAPX-silenced plants strongly supports the possibility that chloroplastic H_2O_2 is involved in the regulation of the SA response. It is possible that chloroplastic H_2O_2 induces the expression of gene(s) involved in the reduction of SA toxicity, though no such gene has yet been identified.

Arabidopsis has two *isochorismate synthase* (*ICS*) genes, *ICS1* and *ICS2*, known to be involved in SA biosynthesis.[12] Garcion et al.[12] reported that both ICS enzymes are located in chloroplasts and ICS1 has a dominant role in the biosynthesis of SA. Furthermore, ICS1, but not ICS2, was highly responsive to a pathogen infection which enhanced levels of SA.[13] Thus, the physiological function of ICS2 is largely unknown. Our previous microarray and quantitative RT-PCR (q-PCR) analyses have revealed that the expression of *ICS2* but not *ICS1* increased in response to tAPX silencing, resulting in enhanced levels of SA.[9] In fact, there was no effect of tAPX silencing on the transcript

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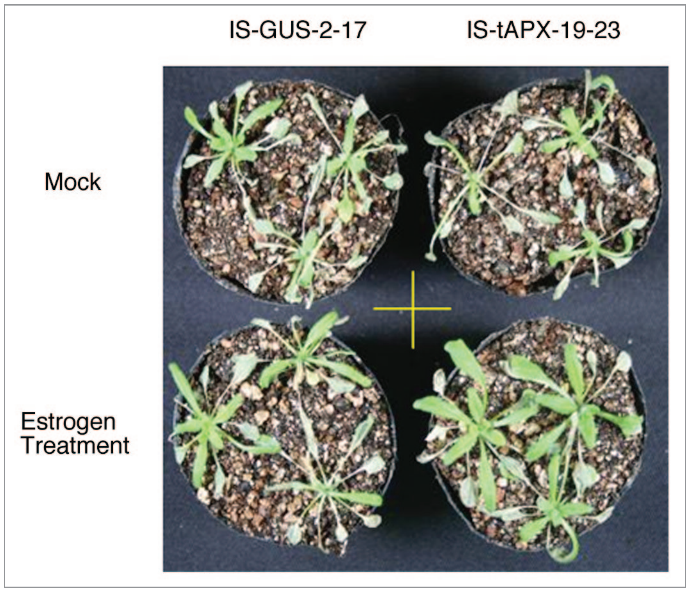

Figure 1. tAPX-silenced plants show SA insensitivity. Plants were grown under continuous light at 100 μmol/m²/s. Seventeen-day-old IS-GUS-2–17 and IS-tAPX-19–23 plants were sprayed with 100 μM estrogen. At 2 d after the treatment, plants were further sprayed with 5 mM SA. The plants 4 d after SA treatment were photographed. The same results were obtained in three independent experiments. A representative photograph is shown.

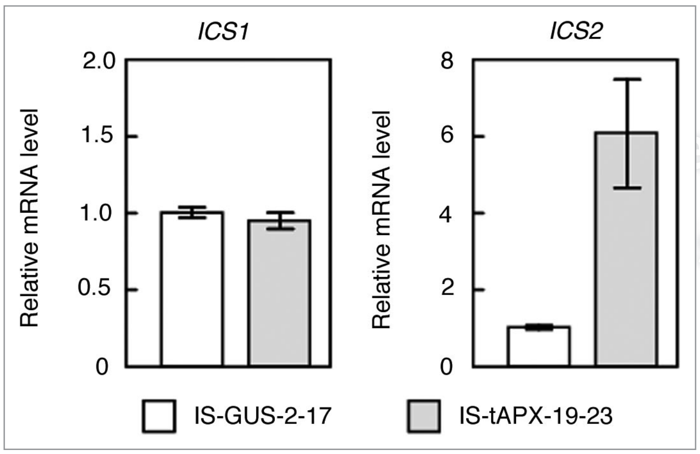

Figure 2. tAPX silencing induces the transcription of *ICS2* but not *ICS1*. Seventeen-day-old IS-GUS-2–17 and IS-tAPX-19–23 plants were sprayed with 100 μM estrogen. At 2 d after the treatment, the transcript levels of *ICS1* and *ICS2* were measured by q-PCR. Error bars indicate SD (n = 3). Significant differences: *, $p < 0.05$ vs. the value for IS-GUS-2–17 plants.

levels of *ICS1* (**Fig. 2**). These findings indicated that chloroplastic H_2O_2 enhances the levels of SA through *ICS2* expression. Mutants lacking *catalase 2*, encoding a major H_2O_2-scavenging enzyme (CAT2) in peroxisomes, have been used to investigate the function of peroxisome-derived H_2O_2.[14] It was found that the CAT2-defective mutants show cell death phenotypes under long-day conditions, and markedly accumulate *ICS1* transcripts and SA.[15] Interestingly, the lack of ICS1 inhibited the accumulation of SA in the mutants and rescued the phenotypes.[15] These findings suggest that peroxisomal and chloroplastic H_2O_2 enhance SA biosynthesis through *ICS1* and *ICS2* expression, respectively.

Taken together, our previous findings and the present results clearly show the role for chloroplastic H_2O_2 in the response to SA. SA acts as an antagonist of abscisic acid (ABA) signaling,[16] which is required plant responses to drought,[17] chilling,[18] and high light.[19] Therefore, it is possible that the negative effect of chloroplastic H_2O_2 on the chilling and high light responses is at least partially due to inhibition of ABA signaling by SA accumulation. Interestingly, comparison of the data from IS-tAPX plants and CAT2-defective mutants suggests a functional difference between peroxisomal and chloroplastic H_2O_2 in regulating the SA response. Analysis of double mutants of IS-tAPX and SA biosynthesis/signaling would reveal the role for the ICS2 pathway in the chloroplastic H_2O_2-mediated stress response, and the physiological significance of source-specific H_2O_2 signaling pathways.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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