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Overexpression of the *CBF2* transcriptional activator in Arabidopsis counteracts hormone activation of leaf senescence

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CBF1-3 **(C-repeat binding factors) are transcriptional activators governing plant responses to low temperatures. Overexpression of *CBF1-3* genes enhances plant frost tolerance, but also causes various pleiotropic effects regarding plant growth and development, mainly growth retardation, and delay of flowering and senescence. In a recent study, we reported that overexpression of *CBF2* suppressed leaf senescence induced by the stress hormone ethylene. Here we show that overexpression of *CBF2* also suppressed chlorophyll breakdown and leaf senescence induced by the phytohormones abscisic acid (ABA), salicylic acid (SA) and methyl jasmonate (MeJA), which indicates its broader role in suppressing hormone-induced leaf senescence. As previously reported for ethylene, the observed decrease in responsiveness to ABA in *CBF2*-overexpressing plants was specific to leaf senescence, since other responses to ABA were similar to those of wild-type plants. Transcript profiling analysis of hormone metabolism and responsive genes revealed that overexpression of *CBF2* induced expression of ABA-biosynthesis and ABA-responsive genes and suppressed SA- and JA-related genes. Overall, in light of the adverse effects of *CBF2* on ABA metabolism and responsiveness, on the one hand, and SA and JA metabolism and responsiveness, on the other hand, we conclude that overexpression of *CBF2* suppresses hormone-induced leaf senescence by directly counteracting the hormone effects on leaf senescence and not by general suppression of their synthesis or signal transduction pathways.**

Overexpression of *CBF2* Suppresses Hormone-Induced Leaf Senescence

CBF1-3 proteins (C-repeat binding factors), also known as DREB1A-C (drought-responsive element binding1), are transcriptional regulators that bind specifically to the C-repeat cis-element present in the promoter regions of cold-responsive genes.[1-3] Ectopic expression of *CBF1* in Arabidopsis induced the expression of cold-regulation genes and significantly enhanced freezing tolerance.[4] In addition to the effects on frost tolerance, overexpression of *CBF1*, *CBF2* and *CBF3* in Arabidopsis also causes various pleiotropic effects on plant growth and development, especially growth retardation, dwarfism, and delayed flowering and leaf senescence.[3,5,6]

Leaf senescence is stimulated by exposure to the plant hormones ethylene,[7] abscisic acid (ABA),[8] salicylic acid (SA)[9] and jasmonic acid (JA).[10] Recently, we reported that overexpression of *CBF2* in Arabidopsis inhibited leaf senescence and chlorophyll breakdown induced by the plant hormone ethylene.[11] In the present paper we show that overexpression of *CBF2* also inhibited chlorophyll breakdown and leaf senescence induced by ABA, SA and MeJA. It can be seen that leaves of wild-type plants turned yellow shortly after incubation in solutions of these hormones, whereas those of the *CBF2*-overexpressing plants stayed green (**Fig. 1A**). Chlorophyll measurements revealed that exposure to ABA, SA and MeJA resulted in major decreases in chlorophyll content in wild-type leaves, to just 62, 65 and 48%, respectively, of

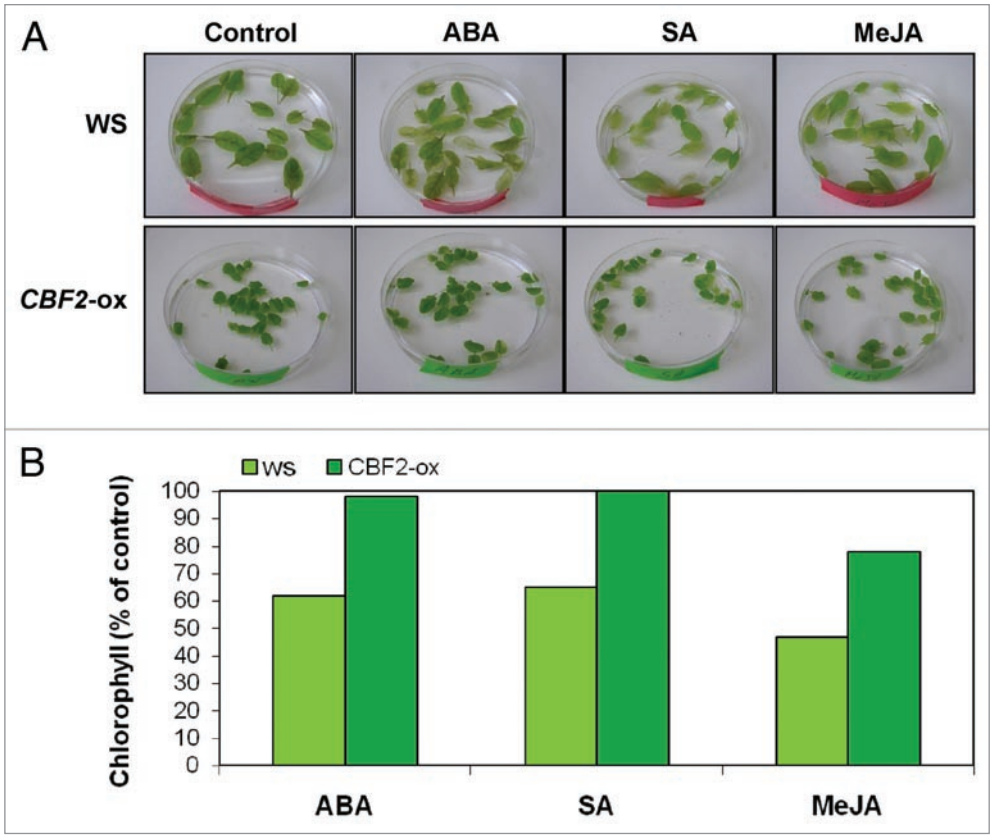

Figure 1. Phytohormone-induced senescence of detached leaves of wild-type (WS2 ecotype), and *CBF2*-overexpressing plants. (A) Photographs of detached leaves after incubation in water (control), ABA (50 μM), SA (100 μM) and MeJA (50 μM). (B) Percentage of chlorophyll content remaining after exposure to the various hormones, as compared with that in leaves incubated in water. Chlorophyll measurements were performed in leaves 5 and 6, detached from rosettes harvested 36 days after sowing. Data in (B) are means of three different experiments, each including four replications.

their contents after incubation in water alone (**Fig. 1B**). In contrast, exposure to ABA, SA and MeJA decreased chlorophyll content to 98, 98 and 75%, respectively of its levels in *CBF2*-overexpressing plants incubated in water alone (**Fig. 1B**).

Effect of *CBF2* on Responsiveness to ABA

In a previous study, we showed that overexpression of *CBF2* specifically counteracted the stimulating effects of ethylene on leaf senescence, but did not affect other plant responses to ethylene: etiolated seedlings of *CBF2*-overexpressing plants responded to ethylene in a similar manner to those of the wild-type.[11] Similarly, we hereby show that overexpression of *CBF2* specifically counteracted the effects of ABA on induction of leaf senescence, but did not affect other responses to the hormone, as increasing concentrations of ABA promoted seed dormancy[12] and inhibited seed germination in a similar manner to that observed in wild type seeds (**Fig. 2**).

Effect of *CBF2* on Transcription of Hormones Metabolism and Responsive Genes

In order to assess the molecular mechanisms involved in governing the responsiveness of *CBF2*-overexpressing plants to ABA, SA and JA, we performed transcript profiling of hormone metabolism and responsive genes, with the Affymetrix ATH1 genome array. By using MapMan[13] software we found that overexpression of *CBF2* induced the expression of ABA-biosynthesis and ABA-responsive genes in leaf tissue but rather suppressed the expression of SA- and JA-related genes (**Fig. 3**). For example, overexpression of *CBF2* induced the expression of 9-cis-epoxycarotenoid dioxygenase, a key enzyme for ABA biosynthesis,[14] and of several other ABA-responsive genes (**Fig. 3**). In contrast, overexpression of *CBF2* suppressed the expression of two SA-biosynthesis genes, and five MeJA-biosynthesis genes, including three lipoxygenases, allene oxide synthase and jasmonic acid carboxyl methyltransferase.[15] Furthermore, overexpression of *CBF2* also suppressed the expression of ATMYC2, a transcription factor involved in mediating JA responses.[16]

Overall, we showed that overexpression of *CBF2* suppressed chlorophyll breakdown and leaf senescence induced by the phytohormones ethylene, ABA, SA and MeJA. In spite of its counteracting effect on leaf senescence, overexpression of *CBF2* did not inhibit other plant responses governed by ethylene and ABA. Furthermore, transcript profiling analysis of hormone metabolism and hormone-responsive genes revealed that overexpression of *CBF2* induced ABA metabolism and ABA-responsive gene expression, but suppressed SA and JA metabolism and

Figure 2. Seed germination of wild-type (WS2 ecotype) and *CBF2*-overexpressing plants in the presence of increasing concentrations of ABA. (A) Photographs of seeds after 7 days growth in the presence of 0, 0.5, 1, 1.5 or 2 μM ABA. (B) Percentage of seed germination in the presence of various concentrations of ABA as compared with that of those grown without ABA. Data in (B) are means ± S.E. of two different experiments, each including 10–20 replications.

signal transduction genes. Therefore, we propose that overexpression of *CBF2* suppresses hormone-induced leaf senescence by directly counteracting the hormone effects on leaf senescence and not by general suppression of their synthesis or signal transduction pathways.

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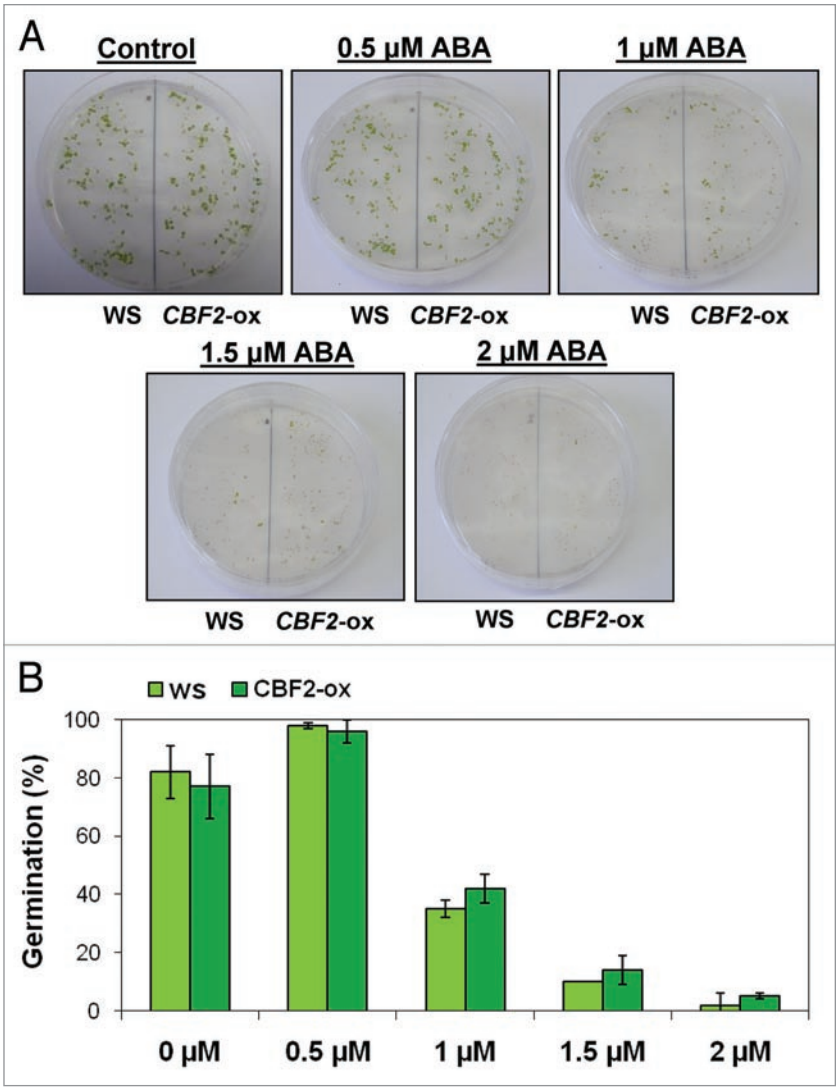

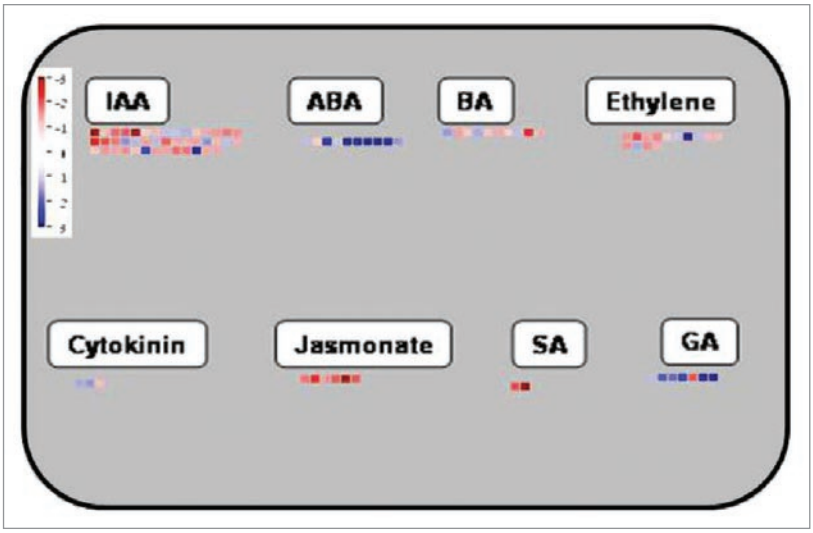

Figure 3. Transcript profiling analysis of hormone metabolism and hormone-responsive genes in mature leaves of *CBF2*-overexpressing plants. Transcripts that were expressed significantly differently in *CBF2*-overexpressing plants ($p \leq 0.05$) on the Affymetrix ATH1 genome array data were categorized by using the MapMan software. Blue color represents upregulated genes and red color represents downregulated genes.

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