DOCTORAL THESIS

# Role and mechanism of abscisic acid in the induction of antioxidant defense in maize leaves

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# Role and Mechanism of Abscisic Acid in the Induction of Antioxidant Defense in Maize Leaves

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# Abstract

Roles and mechanisms of abscisic acid (ABA) in inducing the antioxidant defense were studied in leaves of maize (Zea mays L.) seedlings under water stress and non-stressed conditions.

Treatments with 10 and 100 μM ABA significantly increased the generation of $O_2^{\cdot-}$ and $H_2O_2$, followed by an increase in activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), and the contents of several antioxidant metabolites such as ascorbate (AsA), reduced glutathione (GSH), α-tocopherol (α-TOC) and carotenoid (CAR) in a dose- and time-dependent pattern in leaves of maize seedlings. Under a moderate water stress, pretreatment with 100 μM ABA caused an obvious reduction in the content of catalytic Fe, which is critical for $H_2O_2$-dependent $^{\cdot}OH$ production, and significant increases in the activities of antioxidant enzymes and the contents of non-enzymatic antioxidants, and then significantly reduced the contents of dehydroascorbate (DAsA) and oxidized glutathione (GSSG), which are considered as indices of oxidative stress, and the degrees of oxidative damage to lipids (expressed as MDA content) and proteins (in terms of carbonyl groups) in leaves induced by the moderate water stress. Pretreatment with an ABA biosynthesis inhibitor, tungstate, significantly suppressed the accumulation of ABA induced by water stress, reduced the enhancement in the capacity of antioxidant defense systems, and resulted in an increase in catalytic Fe, DAsA and GSSG, and oxidative damage in the water-stressed leaves. These effects were completely prevented by addition of ABA, which raised the internal ABA content. These data indicate that ABA plays an important role in the induction of antioxidant defense against oxidative stress in plants under water stress and non-stressed conditions.

Time-course analyses of ABA content, the production of reactive oxygen species (ROS), and the activities of antioxidant enzymes in water-stressed leaves showed that a significant increase in the content of ABA preceded that of ROS, which was followed by a marked increase in the activities of these antioxidant enzymes. Pretreatment with an ABA biosynthesis inhibitor, tungstate, significantly suppressed the accumulation of ABA, and also reduced the increased generation of ROS and the up-regulation of these antioxidant enzymes in water-stressed leaves. A mild oxidative stress induced by paraquat, which generates $O_2^{\cdot-}$ and then $H_2O_2$, resulted in a significant enhancement in the activities of antioxidant enzymes in non water-stressed leaves. Pretreatment with some ROS scavengers, such as Tiron and dimethylthiourea (DMTU), and an inhibitor of NAD(P)H oxidase, diphenyleneiodonium (DPI), almost completely arrested the increase in ROS and the activities of these antioxidant enzymes induced by water stress or ABA treatment. These data suggest that water stress-induced ABA accumulation triggers the increased generation of ROS, which in turn leads to the up-regulation of the antioxidant defense system.

Under water stress, the ABA-dependent generation of ROS originates, at least in part, from the plasma membrane NADPH oxidase pathway. Treatments by exogenous ABA or osmotic stress significantly increased the activity of plasma membrane NADPH oxidase and the production of leaf $O_2^{\cdot-}$. Pretreatment with three different inhibitors of NADPH oxidase (diphenylene iodonium, imidazole and pyridine) or an inhibitor of ABA biosynthesis (tungstate) reduced the increase in the activity of plasma membrane NADPH oxidase and the production of leaf $O_2^{\cdot-}$, and the capacity

of antioxidant defense systems mediated by ABA. The inhibitory effects above caused by tungstate were reversible by exogenously applied ABA. These data indicate that NADPH oxidase is involved in the ABA- and water stress-induced production of ROS and antioxidant defense systems, and water stress-induced ABA accumulation plays an important role in the regulation of NADPH oxidase activity.

$Ca^{2+}$ was also shown involved in the ABA signal transduction in plants. The increases in the activity of NADPH oxidase, the production of ROS, and the capacity of antioxidant defense induced by ABA were blocked by the pretreatment with $Ca^{2+}$ chelator EGTA or $Ca^{2+}$ channel blockers $La^{3+}$ and verapamil. Treatment with $Ca^{2+}$ also significantly induced the increases in NADPH oxidase activity, $O_2^-$ production and the activities of the antioxidant enzymes, and the increases were arrested by the pretreatment with the NADPH oxidase inhibitors. The up-regulation in the activities of the antioxidant enzymes induced by oxidative stress by paraquat was suppressed by the pretreatment of $Ca^{2+}$ chelator and $Ca^{2+}$ channel blockers. Our data indicate that a cross-talk between $Ca^{2+}$ and ROS that originate from plasma membrane-bound NADPH oxidase is involved in ABA signal transduction pathway leading to the induction of antioxidant enzyme activity, and $Ca^{2+}$ functions in the upstream as well as downstream of ROS production in the signal transduction event in plants, suggesting the existence of intracellular networks rather than linear pathways in ABA signal transduction leading to the induction of antioxidant defense in plants.

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