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Changes in Antioxidative Enzymes of Young and Mature Leaves of Tomato Seedlings under Drought Stress

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Abstract: The effects of exogenous abscisic acid (ABA) on antioxidant enzymes of young and mature leaves of 4-week old *Lycopersicon esculentum* cv. Ailsa Craig and ABA-deficient mutant, *notabilis*, were investigated under drought stress. Although ABA induced increases in ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) activities in drought-stressed young leaves of *notabilis*, CAT activities remained unchanged in both ABA-treated young and mature leaves of Ailsa Craig under drought. Superoxide dismutase (SOD) activity did not substantially increase in *notabilis* or Ailsa Craig in all treatments. APX activity significantly increased in ABA-, drought- and drought+ABA- treated young leaves of *notabilis*. These results indicated that the antioxidant enzyme activities in drought-stressed and ABA-treated leaves might change with the production of active oxygen species (AOS) depending on the ABA content, and the developmental stages of leaves might contribute to the differential prevention of oxidative damage in plants exposed to drought.

Key Words: Abscisic acid, Antioxidant enzymes, Drought stress, *notabilis* mutant, *Lycopersicon esculentum*

Kuraklık Stresi Altındaki Domates Fidelerinin Genç ve Olgun Yapraklarının Antioksidant Enzimlerindeki Değişiklikler

Özet: Kuraklık stresine maruz bırakılan 4 haftalık *Lycopersicon esculentum* Mill. cv. Ailsa Craig (AC) and absisik asit (ABA) mutantı *notabilis*'in genç ve olgun yapraklarında antioksidant enzimler üzerine ABA uygulamasının etkisi incelendi. ABA kuraklık stresi uygulanan *notabilis*'in genç yapraklarında askorbat peroksidaz (AP), glutatyon reduktaz (GR) ve katalaz (KAT) aktivitelerini arttırırken, Ailsa Craig'in genç ve olgun yapraklarında KAT aktivitesi değişmemiştir. Bütün uygulamalarda *notabilis* ve Ailsa Craig'de superoksit dismutaz (SOD) aktivitesinde belirgin bir değişiklik olmamıştır. *Notabilis*'in ABA, kuraklık ve kuraklık-ABA uygulanan genç yapraklarında APX aktivitesi belirgin bir şekilde artmıştır. Bu sonuçlar kuraklık stresi ve ABA uygulanan yapraklarda antioksidant enzim aktivitelerinin ABA içeriğine bağlı olarak üretilen aktif oksijen türleri (AOT) ile değişebileceğini ve yaprakların gelişim evrelerinin kuraklığa maruz bırakılan bitkilerde oksidatif hasarın farklı şekilde önlenmesine katılabileceğini göstermektedir.

Anahtar Sözcükler: Absisik asit, Antioksidant enzimler, Kuraklık stresi, *notabilis*, *Lycopersicon esculentum*

Introduction

In the last decade, the role of the hormone abscisic acid (ABA) in the induction of antioxidant defense has been the subject of extensive research (1-3). Stress responses of the roots and shoot tissues appear to be coordinated by increased amounts of hormones moving in the xylem sap by root-to-shoot communication (4). It has been documented that ABA can result in the increased generation of active oxygen species (AOS) (1,2,5), induce the expression of antioxidant genes encoding superoxide dismutase (SOD; EC.1.15.11) and catalase (CAT; EC.1.11.1.6) (1,2,6) and enhance the activities of antioxidant enzymes such as SOD, CAT, ascorbate peroxidase (AP; EC.1.11.1.11) and glutathione reductase (GR; EC.1.6.4.2) (2). ABA appears to mediate physiological processes in response to osmotic stress. Levels of endogenous ABA increase in tissues subjected to osmotic stress due to desiccation (7,8). However, details about the interaction between ABA, AOS and antioxidant response remain to be determined. AOS are partially reduced forms of atmospheric oxygen. They typically result from the excitation of O_2 to form singlet oxygen (O_2^1) or from the transfer of 1, 2 or 3 electrons to O_2, respectively, a superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or a hydroxyl radical ($OH^{\cdot}$). The cells are normally protected against AOS by the operation of the

antioxidant defense system comprising enzymic (SOD, CAT, GR, APX) and nonenzymic (ascorbate, α-tocopherol, carotenoids, glutathione) components (9-11). AOS production is further enhanced during exposure to various abiotic stresses, such as drought (12,13) and salt (14-16). The activities of enzymes of the antioxidant system in plants under stress are usually regarded as an indicator of the tolerance of genotypes against stress conditions.

ABA-deficient mutants are used to address the function of ABA in root-stressed plants. These genotypes have been proven useful in clearly identifying ABA as an important factor in regulating whole plant responses to drought (17).

In the present study we utilized such an approach with *Lycopersicon esculentum* Ailsa Craig and ABA-deficient mutant (*notabilis*) genotypes of tomato. The *notabilis* mutant is deficient in ABA and is phenotypically wilty, stunted and epinastic (8). The *notabilis* mutant was reported to have a similar leaf ABA concentration to Ailsa Craig under non-stressed conditions, but accumulated very little ABA in response to leaf dehydration, compared with Ailsa Craig (4,18).

The aim of this study was to identify the effects of ABA on the antioxidant enzymes in mature and young leaves in tomato and ABA-deficient mutant (*notabilis*) under drought stress.

Materials and Methods

Plant material and stress application

The genotypes studied were *Lycopersicon esculentum* Mill. cv. Ailsa Craig (AC) and its ABA-deficient mutant, *notabilis* (seeds obtained from the Tomato Genetic Resource Center, Department of Vegetable Crops, University of California, Davis, California, USA). Uniform seeds were imbibed in aerated water for 1 day at 25 °C. Then they were transferred to plastic pots filled with a sand:soil:manure mix (2:2:1, 3000 g). Plants were grown at 25/20 ºC day/night temperature, and 75 ± 5 % relative humidity in a growth chamber with 480 $\mu mol.m^2s^{-1}$ light (day/night 12/12 h).

Drought treatment was initiated 4 weeks after imbibition. For the control treatment, a set of plants was irrigated daily. For induced drought treatments, a set of plants was not irrigated. ABA (±) was obtained from Sigma Chemical Co. ABA solution (10^{-5} M) was applied 3 times at 1-day intervals by spraying on the growing leaves of half of stressed and unstressed plants (19). Untreated plants were sprayed with distilled water. ABA-treated plants were compared with untreated plants at the same stress level. Samples from leaves of different age from the top (3rd, young leaves, and 9th, mature leaves) were collected 6 days after the last ABA application. Distinct phenotypic differences between young and mature leaves were not observed in well-watered control or droughted plants.

Enzyme extraction and assay

Washed fresh leaves (1 g) were homogenized in 5 ml of 0.1 M potassium phosphate buffer (pH 6.8) containing 0.1 mM EDTA and 100 mg of polyvinyl pirolidone. The homogenate was centrifuged at 15,000 g for 20 min at 4 ºC and the supernatant was immediately used for the following enzyme assays. Total SOD activity was assayed by monitoring the inhibition of the photochemical reaction of nitro blue tetrazolium (NBT) according to the method described by Beyer and Fridowich (20). One unit of SOD activity was defined as the amount of enzyme that was required to cause 50 % inhibition of the reduction of NBT as monitored at 560 nm. CAT activity was assayed by measuring the rate of decomposition of H_2O_2 at 240 nm, as described by Aebi (21). GR activity was measured by following the change in 340 nm as oxidized glutathione (GSSG)-dependent oxidation of NADPH, according to the method given by Carlberg and Mannervik (22). To determine APX activity, fresh leaf tissue (1 g) was homogenized in 15 ml of extraction medium containing 200 mM HEPES, 2 mM EDTA, 5 mM $MgCl_2$, and 4 mM sodium ascorbate. The crude extract was centrifuged at 16,000 g for 5 min at 4 ºC, and the supernatant was used for the measurements. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7), 500 mM ascorbate, 1 mM H_2O_2 and extract. A fall in absorbance at 290 nm was measured as ascorbate was oxidized. APX activity (U g^{-1} FW) was calculated using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$ for ascorbate at 290 nm (23,24).

Statistical Evaluation

The data of 6 measurements from 3 independent experiments were compared using 2 component (age, treatments) analysis of variance. Statistical significance was assessed at the $P < 0.05$ level using 3 way ANOVA

(SPSS 11.5 software package). Means and standard error were calculated from 3 replicates. In all the figures, the spread of values is shown as error bars representing standard errors of the means.

Results

ABA treatment or drought stress led to changes in the activities of APX, GR and CAT in both young and mature leaves of tomato seedlings (Figures 1-4). There were significant differences between SOD activities of young and mature leaves of AC ($P < 0.01$). Mature leaves showed higher SOD activity than young leaves in AC. However, ABA and/or drought treatments caused only negligible changes in activities of SOD in leaves of AC. In contrast, this enzyme increased remarkably in the mature leaves of *notabilis* exposed to drought+ABA (Figure 1). Differences between SOD activities of AC and *notabilis* were significant ($P < 0.05$).

There were significant differences in APX activities in age, treatments between AC and *notabilis* ($P < 0.001$). Drought or ABA treatment decreased APX activities in both young and mature leaves of AC, compared with the control. This enzyme's activities strongly increased in ABA-, drought- and drought+ABA-treated young leaves of *notabilis*, but not in mature leaves ($P < 0.05$) (Figure 2).

The differences between young and mature leaves in GR activities were statistically significant in AC and *notabilis* ($P < 0.05$). The activities of GR remained substantially unchanged with ABA treatments in young and mature leaves of AC, while enzyme activity decreased in ABA-treated young leaves of *notabilis* (Figure 3). Drought induced an increase in the activity of GR in young leaves of both genotypes. However, exogenous ABA significantly increased GR activity in young leaves of *notabilis* under drought, compared with the control and drought ($P < 0.05$).

The activity of CAT remained close to control levels while it increased in young and mature leaves of ABA-treated AC during drought (Figure 4). Exogenous ABA significantly increased CAT activity in the droughted

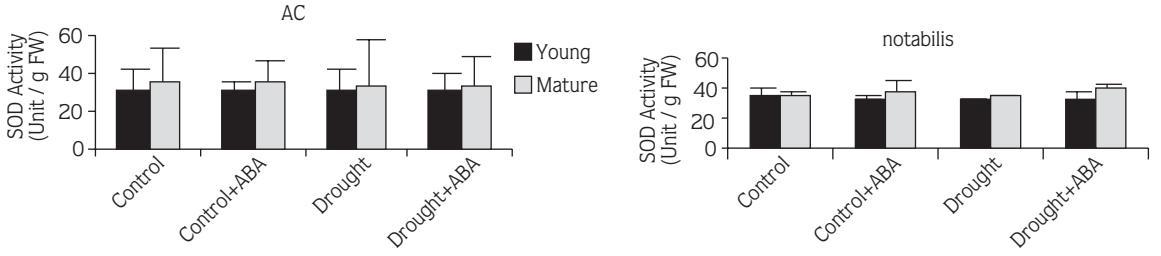

Figure 1. Effects of drought and exogenous ABA on the SOD activity in the leaves of *notabilis* and *L. esculentum* Mill. Ailsa Craig. Values are means ± SE (n = 3).
C; control, C+A; control+ABA, D; drought, D+A; drought+ABA.

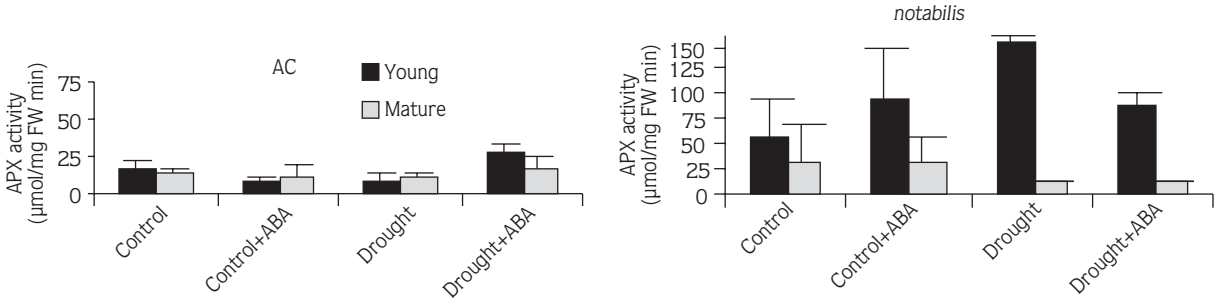

Figure 2. Effects of drought and exogenous ABA on the APX activity in the leaves of *notabilis* and *L. esculentum* Mill. Ailsa Craig. Values are means ± SE (n = 3).
LSD = 33,854

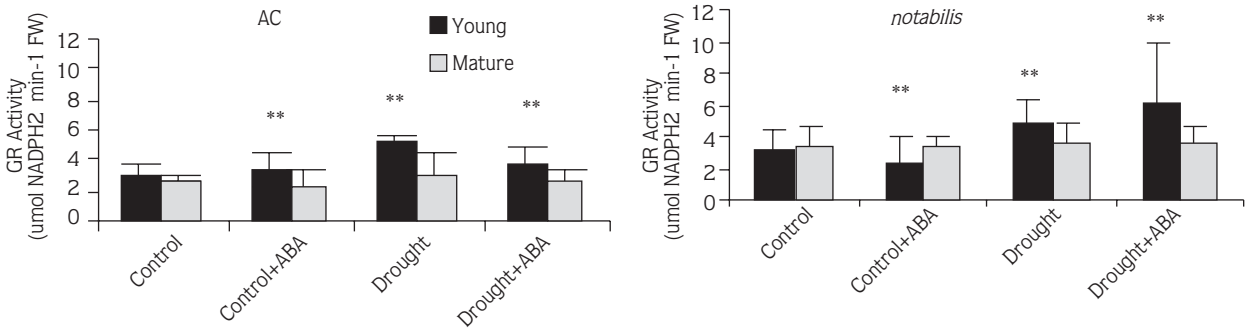

Figure 3. Effects of drought and exogenous ABA on the GR activity in the leaves of *notabilis* and *L. esculentum* Mill. Ailsa Craig. Values are means ± SE (n = 3). **P < 0.01 compared with the control.
LSD = 1.092

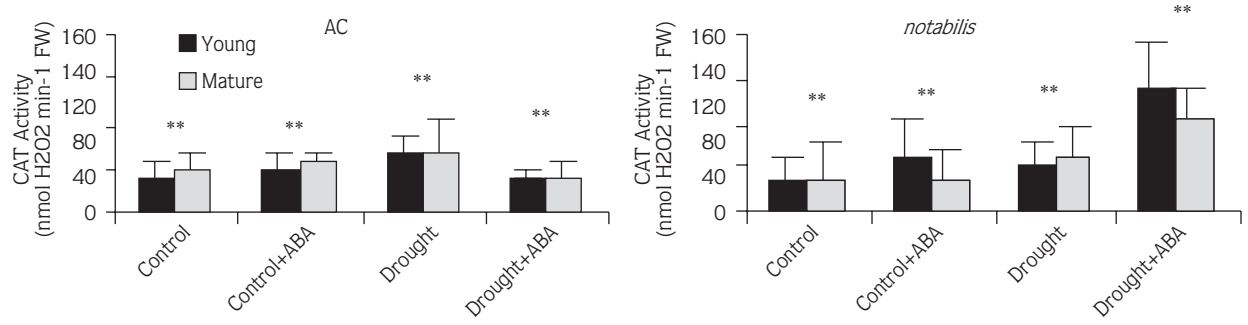

Figure 4. Effects of drought and exogenous ABA on the CAT activity in the leaves of *notabilis* and *L. esculentum* Mill. Ailsa Craig. Values are means ± SE (n = 3). **P < 0.01 compared with the control.
LSD = 21,149

notabilis (P < 0.01). In the young leaves, the activity of CAT was higher than that of ABA-treated mature leaves of *notabilis* exposed to drought. The differences between the treatments in *notabilis* and AC were statistically significant (P < 0.05).

Discussion

The results obtained in the present study show that ABA can vary the activities of antioxidant enzymes in response to drought stress. ABA-mediated metabolic changes lead to changes in oxygen free radical levels, which, in turn, lead to induction of the antioxidant defense system (1,2,25). Enzymatic activities of the Halliwell-Asada Pathway have been separately correlated with different stress situations and the balance between the formation and detoxification of AOS is critical to cell survival during periods of water stress (26).

The important components of protective systems are enzymatic defenses such as SOD, CAT, APX and GR, which scavenge O_2^{-}, H_2O_2 and OH^{-} (13,27). In contrast to the mature leaves of *notabilis*, where drought+ABA treatment increased SOD activity, this enzyme activity remained substantially unchanged with drought and/or ABA treatments in young leaves of both tomatoes. Some researchers have suggested that drought and ABA treatments enhanced SOD activity by increasing H_2O_2 in the mature leaves (2,25). Hence, exogenous ABA is a regulator factor for the effective scavenging of AOS in ABA-deficient mutant *notabilis*. Other researchers also reported that ABA treatment or drought increased the activity of SOD in tobacco cell cultures (26), mature leaves of Arabidopsis (29), leaves of wheat (30), maize (2) and sunflower (19). In our previous studies, we observed that exogenous ABA also induced structural responses to water stress in leaves of *notabilis*. ABA

increases xylem differentiation to increase water flow in xylem in *notabilis* exposed to ABA under water stress (28).

GR and CAT activities increased in drought-stressed young leaves of AC and *notabilis*, whereas ABA treatment significantly increased these enzyme's activities in drought-stressed leaves of *notabilis*. This is in agreement with the results of other researchers who showed that exogenous ABA increased CAT activities in young maize leaves (1) and tobacco (3). It could be considered a response to ABA- and drought-induced oxidative damage, suggesting enzymatic removal of H_2O_2 by CAT. However, GR activity appears not to be significantly influenced by stress, and increases in total GR activity in stressed plants were never found to be greater than 2.5-fold under any of the experimental conditions tested (26). In other studies, SOD, APX and GR activities have been reported to increase during drought stress in mature leaves of Arabidopsis (29). Increased APX activity in drought-stressed young leaves of *notabilis* would increase the demand for ascorbate generation mediated through increased GR activity.

Exogenous ABA strongly enhanced the antioxidant enzyme activities of young leaves under drought stress, especially in *notabilis*. This showed that the enzymatic antioxidants are not predominantly responsible for controlling free radical-dependent damage in ABA-treated mature leaves exposed to drought stress.

The data presented in this paper suggest that the enzymatic antioxidants of tomato leaves are regulated not only by stress and ABA but also by the developmental stages of the tomato leaves.

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