

**ANTIOXIDANT METABOLISM IN WATER STRESSED PEANUT
TREATED WITH DINICONAZOLE**

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ABSTRACT

The response of alpha-tocopherol, glutathione and ascorbate was studied in peanut (*Arachis hypogea* L., cv. NC-17) subjected to water stress and treated with a triazole fungicide, diniconazole (DINI). There was no significant difference in alpha-tocopherol levels between control and DINI treated plants. However, a 14% increase was observed in alpha-tocopherol level in DINI treated, water stressed plants compared to water stressed plants. Total glutathione in DINI treated stressed plants increased by 13 and 31% over control and water stressed plants, respectively. Ascorbate levels decreased significantly in all treatments compared to the control. These results indicate that DINI alleviates the oxidative damage caused by water stress by increasing total glutathione levels. However, DINI does not seem to affect alpha-tocopherol and ascorbate levels in peanuts under water stress.

INTRODUCTION

Protection against harmful oxidation of biomembranes is provided by several antioxidative systems, including a large array of enzymes and a variety of small molecules such as glutathione, beta-carotene, and the vitamins C and E (Finckh and Kunert 1985). In plants, vitamin E (alpha-tocopherol) is located mainly in the chloroplast (Bucke 1976) and a high concentration of the lipid-soluble vitamin is found in the chloroplast envelope (Lichtenthaler *et al.* 1981). Vitamin C (ascorbate), a reducing agent, is present in substantial amount in higher plants (Jones and Hughes 1983), and the water-soluble vitamin is generally regarded as a normal cellular constituent (Finckh and Kunert 1985).

The tripeptide glutathione is widely distributed in plant cells (Rennenberg 1982). It is implicated in the adaption of plants to environmental stress such as drought and extremes of temperature (Alscher 1989).

Glutathione, ascorbate and alpha-tocopherol are of special interest because of their demonstrated association with stress resistance. The present study evaluated the response of alpha-tocopherol, glutathione and ascorbate in water stressed peanut plants treated with diniconazole.

MATERIALS AND METHODS

Peanut (*Arachis hypogea* L., cv. NC-17) seedlings were grown in controlled conditions, i.e. continuous illumination (392 uEm-2sec-1) and controlled temperature ($28 \pm 2^\circ\text{C}$). Treatments consisted of control, water stressed, DINI treated and DINI treated stressed plants arranged in a completely randomized block design with 10 replications. DINI was applied foliary at a rate of 4 mg/plant (recommended field rate) every 2 weeks for 7 weeks commencing when the plants were 6 weeks old. Drought stress treatment began 4 days following initial DINI treatment and consisted of applying 125 ml of distilled water to plants subjected to drought every 3 days versus 250 ml to non-stressed plants. Harvested plants (117 days old) were frozen in liquid nitrogen, freeze dried and stored at -23°C until analyzed for alpha-tocopherol, glutathione and ascorbate.

Alpha-tocopherol was extracted in 80% ethanol partitioned with hexane and the hexane layer analyzed using a Hewlett Packard Model 1090 HPLC, equipped with a fluorescence detector (294 nm excitation, 325 nm emission) following modified procedures of Cort *et al.* (1983) and Grumbach (1983). The hexane ran through a silica column (Hypersil 5 um, 200 x 4.6 mm, Hewlett Packard).

Glutathione and ascorbate extraction was accomplished by homogenizing leaf tissue in 2% metaphosphoric acid. The homogenate was centrifuged at 17 000 g and the supernatant analyzed for glutathione (Griffith 1980) and ascorbate (Foyer and Halliwell 1977) using a Beckman DU-65 spectrophotometer at wavelengths 412 nm and 523 nm, respectively.

RESULTS AND DISCUSSION

The following table reflects the responses of alpha-tocopherol, glutathione and ascorbate in water stressed peanut plants treated with DINI.

Alpha-tocopherol, total glutathione and ascorbate levels in water stressed peanut (*Arachis hypogea* L., cv. NC-17) plants treated with diniconazole

TRT	VITAMINE (% DW)	GLUTATHIONE (nMoles/gDW)	VITAMIN C (uMoles/gDW)
C	0.57A	835.01B	7.28A
T	0.43B	880.40AB	3.91B
CS	0.49AB	719.86C	4.65B
TS	0.50AB	946.14A	4.74B

C = control; T = treated w/ DINI; CS = water stressed; TS = treated w/ DINI and water stressed. Value with different letters are significantly different at $\alpha = 0.05$ (LSD).

There was no significant difference in alpha-tocopherol levels between control and DINI treated plants. However, a 14% increase was observed in alpha-tocopherol level in DINI treated stressed plants compared to water stressed plants. Earlier reports (Senaratna *et al.* 1985, Mackay *et al.* 1987) on antioxidant levels in stressed plants indicated an increase on the levels of total antioxidants. Mackay *et al.* (1987) evaluated the antioxidant potential of microsomal membranes and reported an increase in antioxidant potential of triazole (S-3307) treated wheat plants exposed to ozone. However, their analysis was made on total antioxidant potential of the lipid fraction, as the ability of the lipid extract to inhibit the *in vitro* oxidation of exogenous linoleic acid. The oxidation reaction was similar to that seen for alpha-tocopherol and thus, the antioxidant capacity of their samples were expressed as alpha-tocopherol equivalents (Mackay *et al.* 1987). In our study, alpha-tocopherol was measured directly by HPLC.

Ascorbate levels decreased significantly in all treatments compared to the control. There is evidence that ascorbate represents a reservoir of antioxidant potential to regenerate directly, under conditions of stress, the lipid-soluble primary antioxidant alpha-tocopherol (Packer *et al.* 1979; Leung *et al.* 1981). This could explain the significant decrease in ascorbate levels in water stressed and DINI treated water stressed plants compared to the control.

Total glutathione in DINI treated stressed plants increased by 13 and 31% over control and water stressed plants, respectively. In pea, a chilling-resistant species, total glutathione decreased only slightly, but was still higher than that in cucumber, a chilling-sensitive species (Wise and Naylor 1987). The results presented here indicate that DINI alleviates the oxidative damage caused by water stress by increasing the total glutathione levels.

SUMMARY

Total glutathione levels increased in stressed peanut plants treated with diniconazole and could be a factor in drought resistance.

Diniconazole does not seem to affect alpha-tocopherol and ascorbate levels in peanuts under water stress.

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