Superoxide dismutase and peroxidase activities in drought sensitive and resistant barley (*Hordeum vulgare* L.) varieties

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**Key words:** *Hordeum vulgare* L., superoxide dismutase, peroxidase, water deficit, drought

**Abstract**

The effects of water deficit on relative water content (RWC), on the activity superoxide dismutase (SOD) and peroxidase (POX) from leaves of two drought-resistant barley strains (*Hordeum vulgare* L.) varieties (TOKAK-157/37 and 56000/MISC-233) and one sensitive (ERGINEL-90) were studied.

In 21 day old seedlings, drought stress was initiated by withholding water and lasted for 12 days. Activity of SOD increased by the effect of drought treatments in the leaves of drought-resistant varieties TOKAK-157/37 and 56000/MISC-233 as compared to sensitive variety ERGINEL-90. The drought treatment resulted in a 418 % and 59 % increase in SOD activity in resistant varieties at the end of the 12th day of experimental period. However, an increase in activity of SOD was not accompanied by an increase in activity of POX in drought-resistant TOKAK-157/37 and 56000/MISC-233 except on the 6th day of drought treatment in 56000/MISC-233. In drought-sensitive variety, ERGINEL-90, POX activity did not change throughout drought period.

**List of abbreviations:** SOD (superoxide dismutase, EC 1.15.1.1), POX (peroxidase, EC 1.11.1.7), AP (ascorbate peroxidase, EC 1.11.1.11), BSA (bovine serum albumin), CAT (catalase, EC 1.11.1.6), GR (glutathione reductase, EC 1.6.4.2), PVPP (polyvinylpolypyrrolidone), EDTA (Ethylenediaminetetraaceticacid), NBT (nitroblue tetrazolium), RWC (relative water content), TW (turgid weight), FW (fresh weight), DW (dry weight), PAR (Photosynthetic photon flux density), DAB (Diaminobenzidine-tetrahydrochloride dihydrate), MDHAR (Monodehydroascorbate reductase, EC 1.6.5.4), DHAR (Dehydroascorbate reductase, EC 1.8.5.1)

**Introduction**

When plants are subjected to heat shocks, water deficit, low air hygrometry and salinity that can produce drought effect, leaves are known to close their stomata to prevent further water loss as a major temporary adaptive response. This process reduce the availability of CO₂ for photosynthesis which can lead to the formation of reactive oxygen species from the misdirecting of electrons in photosystems. As a result, reactive oxygen species such as superoxide radical (*O₂−*), hydroxyl radical (*OH*'), hydrogen peroxide (*H₂O₂*) and singlet oxygen (*O₂*) are formed in photosynthetic cells. As they contain high concentration of oxygen, a large amount of putative photosensitizing pigments and polyunsaturated lipids, photosynthetic cells are prone to oxidative stress (Boo and Jung 1999).

To minimize the damaging effects of activated oxygen, plants have evolved various enzymatic and nonenzymatic mechanisms, involving small molecular antioxidants and antioxygenic enzymes that
cope with the formation of toxic oxygen species in a variety of ways (Tsang et al. 1991).

Among antioxidants, superoxide dismutase (SOD) removes $O_2^-$ by catalyzing the dismutation of it into $H_2O_2$ and $O_2$ (Monk et al. 1989, Boo and Jung 1999). The production of $H_2O_2$ can directly be countered by the activities of CAT and nonspecific POX. However, since chloroplasts contain no CAT and little 'nonspecific' POX activity, other enzymatic mechanisms are also required to reduce accumulation and deleterious effects of $H_2O_2$. In chloroplasts, $H_2O_2$ is also eliminated by the action of ascorbate-glutathione cycle where AP, MDHAR, DHAR and GR are the key enzymes (Foyer and Halliwell 1976).

High constitutive or high induced levels of antioxidants in a plant cell may provide resistance to a particular stress (Tanaka and Sugahara 1980, Mishra et al. 1993). From foregoing researches, high SOD activity was linked with drought stress tolerance in plants that survive treatments likely to enhance the production of $O_2^-$ (Bowler et al. 1992 a,b). Increase in the activity was reported in drought stressed tomato (Perl-Treves et al. 1988), in maize (Pastori and Trippi 1993) and in cotton cultivars (Gosset et al. 1994), which differ in sensitivity to water deficit.

On the other hand, Smirnoff and Colombe (1988) reported elevated levels of glutathion reductase and ascorbate peroxidase in drought-tolerant Hordeum species. However, SOD and POX activity was not examined. We presume that a similar kind of induction of antioxidants including SOD and POX biosynthesis might occur in drought-tolerant barley (Hordeum vulgare L.) varieties. Therefore, the present study was carried out to monitor SOD and POX changes in drought-tolerant, TOKAK-157/37 and 56000/MISC-233 and drought-sensitive cultivars, ERGINEL-90 under the effect of drought stress.

Materials and Methods

Plant materials and growth conditions

Two strains of Hordeum vulgare L., one sensitive (ERGINEL-90) and one resistant (TOKAK-157/37) to drought stress were obtained from Bahri Dagdas International Winter Cereals Research Center (B.D. MIKHAM, Konya/TURKEY). Another drought-resistant barley strain (56000/MISC-233) was obtained from E. U. Agriculture Faculty. Seeds were germinated in perlit in a growth chamber under controlled environmental conditions at three independent trials (light/dark regime of 16/8 h at 24/20 °C, relative humidity 60-70 %, photosynthetic photon flux density of (PAR) 350 µmol·m$^{-2}$·s$^{-1}$ at plant height.

Until day 20th, seedlings were grown in perlit and watered with Hoagland nutrient solution (100 %). On the 21st day, one set of plants was not watered (drought treatment) while control plants were watered as before.

For enzyme analysis the first fully expanded leaves were collected at the beginning of the drought treatment, after day 21, and the following 6th, 9th and 12th day of the treatment. The sampling were done at 1500 pm at every sampling time. Leaves that were used for enzyme analysis were frozen in liquid nitrogen immediately after harvesting. These samples were stored at - 40 °C until they were used.

Enzyme Assays

Samples were prepared for SOD, POX and total protein analyses by homogenizing 0.5 g. of frozen leaf material in 3 ml of an cold solution containing $50 \times 10^{-3}$ M Na phosphate buffer (pH; 7.8), $1 \times 10^{-3}$ M EDTA and $2 \%$ (w/v) PVPP. The homogenate was centrifuged at 0 °C for 40 min. at 13000 g.

All spectrophotometric analyses were conducted on a Shimadzu (UV-1601) spectrophotometer.

The SOD activity assay based on the method of Beauchamp and Fridovich (1971), which measure the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of enzyme activity was defined as the quantity of SOD required to produce a 50 % inhibition of reduction of NBT. Total enzyme activity was expressed in units/mg$^{-1}$ protein.

The reaction mixture (3 ml) contained $50 \times 10^{-3}$ M Na phosphate buffer (pH; 7.8), $33 \times 10^{-6}$ M NBT, $10 \times 10^{-3}$ M L-Methionine, $66 \times 10^{-3}$ M EDTA and $33 \times 10^{-6}$ M Riboflavin. Reactions were carried out 10