

Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes

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Abstract

The role of plant antioxidant system in water stress tolerance was studied in three contrasting wheat genotypes. Water stress imposed at different stages after anthesis resulted in a general increase in lipid peroxidation (LPO) and decrease in membrane stability index (MSI), and contents of chlorophylls (Chl) and carotenoids (Car). Antioxidant enzymes like glutathione reductase and ascorbate peroxidase significantly increased under water stress. Genotype C 306, which had highest glutathione reductase and ascorbate peroxidase activity, also showed lowest LPO and highest MSI, and Chl and Car contents under water stress in comparison to susceptible genotype HD 2329, which showed lowest antioxidant enzyme activity as well as MSI, Chl and Car contents and highest LPO. HD 2285 which is tolerant to high temperature during grain filling period showed intermediate behaviour. Thus, the relative tolerance of a genotype to water stress as reflected by its comparatively lower LPO and higher MSI, Chl and Car contents is closely associated with its antioxidant enzyme system.

Additional key words: active oxygen species, ascorbate peroxidase, carotenoids, chlorophylls, glutathione reductase, oxidative injury, oxidative stress, *Triticum aestivum*, water stress.

Introduction

Water stress not only affects cell water potential, induces closure of stomata, and decrease in photosynthesis, nitrate assimilation and various anabolic enzyme reactions (Sairam *et al.* 1990, Sairam 1994), but also induces generation of active oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical (Gamble and Burke 1984, Gillham and Dodge 1987, Cadenas 1989) causing lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Fridovich 1986, Liebler *et al.* 1986, Davies 1987, Imlay and Linn 1988). The detoxification of superoxide radical and hydrogen

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Abbreviations: APO - ascorbate peroxidase; Car - carotenoid; Chl - chlorophyll; DAA - days after anthesis; GR - glutathione reductase; LPO - lipid peroxidation; MDA - malondialdehyde; MSI - membrane stability index.

peroxide consequently is of prime importance in any defence mechanism. Plants possess several tissue antioxidants for protection against the potentially cytotoxic forms of activated oxygen species, such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, glutathione, ascorbic acid, α -tocopherol and carotenoids (Liebler *et al.* 1986, Flstner 1987, Larson 1988).

A tolerant genotype, therefore, should not only be able to retain sufficient water under water stress, but also have highly active system to guard against oxidative injury. The present experiment was, therefore, conducted with three wheat genotypes, widely differing in their response to drought, to study the effect of water stress on glutathione reductase and ascorbate peroxidase in relation to stress tolerance.

Materials and methods

Wheat (*Triticum aestivum* L.) cvs. C 306 (water stress tolerant), HD 2285 (heat tolerant during grain filling period) and HD 2329 (water stress susceptible) were planted in earthen pots of 30 × 30 cm size, filled with sandy loam soil and farm yard manure in 6:1 ratio. Each pot was fertilized corresponding to 120, 60 and 60 kg ha⁻¹ of N, P and K, respectively. Four seedlings were maintained in each pot. Plants were watered when required to keep them fully turgid or water stress was imposed by withholding water supply for 8 d and thereafter irrigated. The first water stress treatment was started at anthesis and the second and the third 10 and 20 d after anthesis. Ten pots per cultivar were placed for each treatment and each stage. Samples from control and treated plants were taken at the end of the treatments (8, 18, 28 DAA).

Leaf relative water content (RWC) was estimated according to the method of Whetherley (1950). Leaf membrane stability index (MSI) was determined according to the method of Premachandra *et al.* (1990) as modified by Sairam (1994). Leaf discs (0.1 g) were thoroughly washed in running tap water and double distilled water and thereafter placed in 10 cm³ of double distilled water at 40 °C for 30 min. After the expiry of the period their electrical conductivity was recorded by conductivity bridge (C₁). Subsequently the same samples were placed in boiling water bath (100 °C) for 10 min and their electrical conductivity recorded as above (C₂). The membrane stability index (MSI) was calculated as:

$$\text{MSI} = [1 - (C_1/C_2)] \times 100$$

Chlorophylls (Chl) and carotenoids (Car) were estimated by extracting the leaf material in 80 % acetone. Absorbance were recorded at 665, 645 and 470 nm. Chl *a*, *b* and total Chl were calculated according to Arnon (1949) and Car content according to Lichtenthaler and Wellburn (1983). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation by the method of Heath and Packer (1968). A leaf sample (0.5 g) was homogenised in 10 cm³ of 0.1 % trichloroacetic acid (TCA). The homogenate was centrifuged at 15 000 g for 5 min. To 1.0 cm³ aliquot of the supernatant 4.0 cm³ of 0.5 % thiobarbituric acid (TBA) in 20 % TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in a ice bath. After centrifugation at 10 000 g for 10 min, the

absorbance of the supernatant is recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The MDA content was calculated by its extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as nmol MDA per gramme of fresh mass.

Enzyme extract for glutathione reductase (GR) and ascorbate peroxidase (APO) was prepared by grinding 0.5 g leaf material with 10 cm^3 of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA, in a prechilled mortar and pestle. The brei was filtered through cheese cloth and the filtrate was centrifuged in a *Beckman* model *J2-21* refrigerated centrifuge for 15 min at 20 000 *g*. The supernatant is referred as enzyme extract. All operations were carried out at 4 °C. GR was assayed by the method of Smith *et al.* (1988). The reaction mixture contained 1.0 cm^3 of 0.2 M potassium phosphate buffer (pH 7.5) containing 1.0 mM EDTA, 0.5 cm^3 of 3.0 mM DTNB (5,5'-dithiobis (2-nitrobenzoic acid) in 0.01 M potassium phosphate buffer (pH 7.5), 0.1 cm^3 of 2.0 mM NADPH, 0.1 cm^3 enzyme extract and distilled water to make up a final volume of 3.0 cm^3 . Reaction was initiated by adding 0.1 cm^3 of 2.0 mM GSSG (oxidized glutathione). The increase in absorbance at 412 nm was recorded at 25 °C over a period of 10 min on a *Beckman* model 36 *UV-VIS* spectrophotometer. The ascorbate peroxidase activity (APO) was assayed according to the method of Nakano and Asada (1981) by recording the decrease in ascorbate content at 290 nm, as ascorbate was oxidized. The reaction mixture contained 1.5 cm^3 of 100 mM potassium phosphate buffer (pH 7.0), 0.5 cm^3 of 3.0 mM ascorbic acid, 0.1 cm^3 of 3.0 mM EDTA, 0.2 cm^3 of 1.5 mM H_2O_2 and 0.1 cm^3 of diluted enzyme in a total volume of 3.0 cm^3 . The reaction was started with the addition of H_2O_2 and absorbance was recorded at 290 nm spectrophotometrically for 5 min.

Results

Relative water content (RWC) in leaves of wheat cultivars under irrigated and stress conditions (Fig. 1) showed a decreasing trend with age in all the genotypes. There was

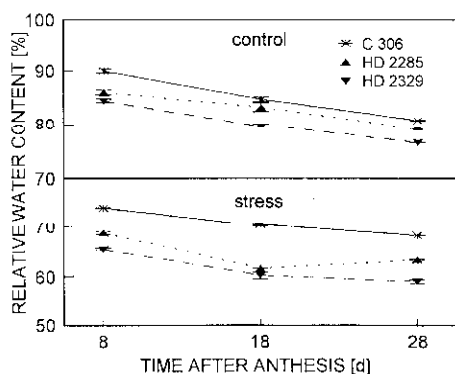


Fig. 1. Effect of water stress on relative water content in wheat genotypes. Bars indicate SE. Differences between genotypes, stages and their interaction were significant ($P = 0.05$).

significant reduction in RWC under water stress in all the cultivars. Genotype C 306 maintained significantly higher RWC both under water stress and irrigated conditions, HD 2285 exhibited medium behaviour, while HD 2329 showed lowest value under water stress. Membrane stability index (Table 1) decreased with increasing age as well as under water stress in all genotypes. Under water stress condition the first stage samples were damaged and, therefore, the data reported is for second and third stages only. MSI was highest in C 306 and lowest in HD 2329, while HD 2285 showed medium behaviour at the two stages under water stress. The genotypic response was almost identical under control condition.

Table 1. Effect of water stress on membrane stability index [%] in wheat genotypes measured at 18 and 28 DAA.

Genotype	Control		Water stress	
	18 DAA	28 DAA	18 DAA	28 DAA
C 306	78.60	71.80	66.02	58.80
HD 2285	79.60	68.75	63.80	52.50
HD 2329	77.13	60.88	53.45	41.85
LSD ($P = 0.05$)				
genotype	1.91		0.99	
stage	1.91		0.81	
genotype \times stage	3.30		1.40	

Lipid peroxidation (LPO) as MDA content, estimated at 8 DAA, showed marked increase under water stress (Table 2). Under control condition HD 2285 showed minimum LPO while HD 2329 showed maximum value. Under drought maximum LPO was observed in HD 2329, however, minimum value was observed in C 306 while HD 2285 showed intermediate behaviour.

Table 2. Effect of water stress on lipid peroxidation [nmol(malondialdehyde) g^{-1} (f.m.)] in wheat genotypes. LSD ($P = 0.5$) were 3.72, 3.04 and 5.27 for genotype, water status and genotype \times water status, respectively.

Genotype	Control	Water stress
C 306	126.88	204.30
HD 2285	118.28	249.46
HD 2329	165.59	279.59

Total chlorophyll (Chl) and carotenoid (Car) content showed decreasing trend with age both under control and stress conditions (Fig. 2). Under irrigation HD 2285 had higher concentrations of both the pigments than C 306 and HD 2329. There was marked reduction in Chl and Car content under water stress at all stages in all cultivars. However, C 306 was able to maintain highest Chl and Car content followed by HD 2285 and HD 2329 under water stress.

There was 50 to more than 100 % increase in glutathione reductase (GR) activity under water stress in comparison to control plants (Fig. 2). In control plants GR activity at 8 and 18 DAA was highest in C 306 while at the last stage (28 DAA) it was highest in HD 2285. HD 2329 had lowest GR activity at all stages. Under stress,

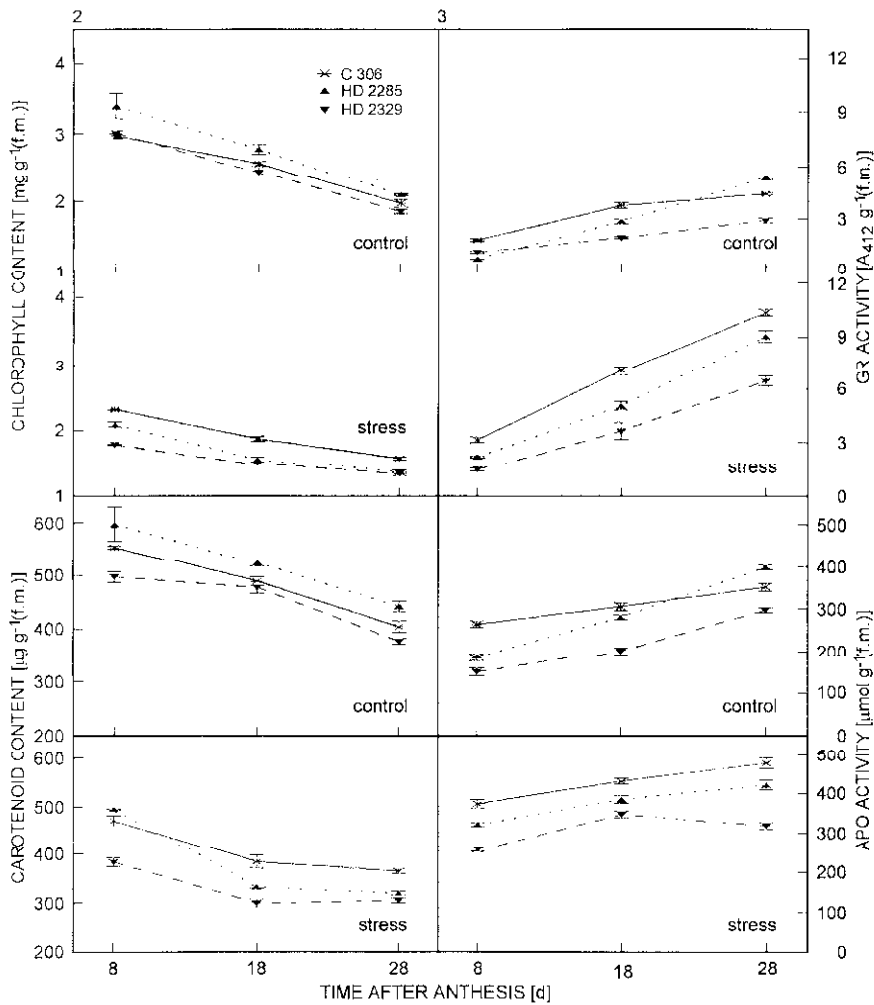


Fig. 2. Effect of water stress on total chlorophyll and carotenoid content, and glutathione reductase and ascorbate peroxidase activity in wheat genotypes. Bars indicate average \pm SE. Differences between genotypes, stages and their interaction were significant ($P = 0.05$).

however, C 306 maintained highest GR activity at all stages, followed by HD 2285 and HD 2329 in decreasing order. Ascorbate peroxidase activity (APO) showed significant increase under water stress as well as with age in all the cultivars at all stages except in HD 2329 which showed decline in activity at 28 DAA (Fig. 2). C 306 exhibited highest

APO activity under water stress and irrigated conditions, followed by IID 2285 and HD 2329 in decreasing order.

Discussion

Differential response of genotypes of same species to environmental stresses provides us with an important tool to have an insight into the physiological mechanisms operative in stress tolerant genotypes. C 306 which is recommended for rainfed cultivation, showed comparatively higher RWC and membrane stability, and lower lipid peroxidation under water stress in comparison to susceptible genotype HD 2329 (recommended for irrigated condition), while HD 2285 which is tolerant to high temperature during grain filling, showed intermediate response. Decrease in membrane stability reflects the extent of lipid peroxidation caused by active oxygen species (Dhindsa *et al.* 1981, Dhindsa 1991). Premachandra *et al.* (1990) has reported that cell membrane stability is an indicator of drought tolerance. Lower lipid peroxidation and higher RWC and membrane stability (lower ion leakage) has also been reported in tolerant genotypes of maize (Pastori and Trippi 1992) and wheat (Kraus *et al.* 1995).

Though under irrigation both Chl and Car contents were highest in IID 2285, which may reflect its relative tolerance to increasing temperature during maturity, however, under water stress C 306 had comparatively higher total Chl and Car contents, signifying lower pigment bleaching in this genotype than in HD 2329 and HD 2285 under stress. Carotenoids are responsible for scavenging of singlet oxygen (Knox and Dodge 1985), hence their comparative levels in a genotype will determine its relative tolerance. Higher Chl and Car content in tolerant genotypes have also been reported earlier (Pastori and Trippi 1992, Sairam 1994, Kraus *et al.* 1995).

Cultivar C 306 which had comparatively higher RWC, MSI, Chl and Car contents and lower LPO also showed higher GR and APO activity as compared to HD 2329 and HD 2285 under water stress as well as under irrigation at most of the stages. GR and APO are involved in the scavenging of the products of oxidative stress, such as hydrogen peroxide generated in chloroplast (Gamble and Burke 1984, Gillham and Dodge 1986, Moran *et al.* 1991, Jagtap and Bhargava 1995), and thus help in ameliorating the adverse effects of oxidative stress. Elevated GR activity during stomatal closure in response to water stress may also serve to ensure the availability of NADP to accept electrons derived from photosynthetic electron transport, thereby directing electrons away from oxygen and minimizing the production of superoxide radicals (Egneus *et al.* 1975, Foster and Hess 1982).

Increased GR activity in drought tolerant genotypes of maize (Pastori and Trippi 1992), tomato (Walker and McKersie 1993), tobacco (Van Rensburg and Kruger 1994) and wheat (Kraus *et al.* 1995) have also been reported. Higher APO activity has also been correlated with relative tolerance of the crop plants (Gillham and Dodge 1987, Kraus *et al.* 1995, Jagtap and Bhargava 1995).

Comparatively higher GR and APO activity and membrane stability, and lower lipid peroxidation in HD 2285 at later stage (28 DAA) under irrigation apparently accounts for its high temperature tolerance during grain filling period.

Thus, it appears that relative tolerance of a genotype as reflected by its lower lipid peroxidation, and higher membrane stability and pigment concentration is related with the levels of its antioxidant enzymes activity (Malan *et al.* 1990, Kraus *et al.* 1995, Jagtap and Bhargava 1995). As part of this system GR plays a key role by increasing its activity, which results in the maintenance of high GSH/GSSG ratio (Gamble and Burke 1984). The increased activities of antioxidant enzymes act as damage control system and thus provide protection from oxidative stress resulting in lower lipid peroxidation and higher membrane stability, chlorophyll and carotenoid content in tolerant genotypes.

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