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SHORT COMMUNICATION

HIGH TEMPERATURE INDUCED ANTIOXIDATIVE DEFENSE MECHANISM IN SEEDLINGS OF CONTRASTING WHEAT GENOTYPES

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Leaf discs of 15 d old seedling of wheat genotypes C-306 (temperature tolerant) and HD 2329 (widely adapted) were incubated at 25, 35 and 45ºC to analyse the extent of membrane injury and antioxidative defense mechanisms. It is suggested that the tolerant genotype C-306 exhibited lower accumulation of MDA and H_2O_2 content owing to increased activities of superoxide dismutase, peroxidase and catalase under high temperature conditions. The higher water retension capacity and lower membrane injury in C-306 further helped it in imparting high temperature tolerance. The HD 2329 was also able to resist high temperature stress to some extent via above adjustments.

***Key words*: Antioxidants, lipid peroxidation, membrane injury, *Triticum aestivum*.**

Drought, salinity and high temperature are among the most limiting abiotic factors affecting wheat production under fragile arid ecosystem. It has been well reported that much of the injury to plants caused by stress exposure is associated with odixative damage at cellular level (Allen 1995). The superoxide radicals and their dismutation product, hydrogen peroxide, can directly attack membrane lipids and inactivate SH-containing enzymes (Sairam *et al.* 2000). The hydroxyl radical, one of the most reactive toxic oxygen species, is responsible for oxygen toxicity *in vivo*, causing damage to DNA, proteins, lipids, chlorophyll and almost every other organic constituent of the living cell (Becana *et al.* 1998). Plants protect cellular and sub cellular system from the cytotoxic effects of these active oxygen radicals with antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase as well as metabolites such as glutathione, ascorbic acid, tocopherol and carotenoids (Alscher *et al.* 2002). It is hypothesized that modulation of the activities of theses enzymes at early growth stage may be important in imparting resistance to a plant against environmental stresses. Therefore, in present investigation the relative significance of antioxidative enzymes and membrane permeability has been examined at early stage in temperature tolerant and susceptible wheat genotypes.

Seeds of wheat genotypes C-306 (drought tolerant) and HD 2329 (temperature sensitive but widely adapted) of uniform size were selected and surface sterilized with 0.1 percent mercuric chloride solution for 2 min and then washed thoroughly with sterilized distilled water. The seeds were sown in small pots available in growth room at day/night average temperature of 25/18±1ºC, RH 60-80% and maximum/minimum PAR of 352/103 µmol m^{-1} s^{-1}. Both fluorescent and candescend lights were made available in the growth room. Cells of these root trainers were filled with loamy sand soil having bulk density 1.48 g cm^{-3}, pH 8.4, field capacity 11.8% and permanent wilting point 2.8%. Leaf discs (5g, 1 cm diameter)were collected from 15 days old wheat seedlings of both the genotypes. These leaf discs were divided into three parts and then were incubated for 2 h at 25ºC (room temperature), 35ºC (moderate temperature and 40ºC (high temperature). These samples were used for the estimation of relative water content (Barrs and Weatherly 1992), membrane injury (Deshmukh *et al.* 1991), superoxide

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dismutase activity (Dhindhsa *et al.* 1981), catalase activity (Teranishi *et al.* 1974) and peroxidase activity (Castillo *et al.* 1984). The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content (Heath and Packer 1968) whereas H_2O_2 content was estimated using the method of Mukherjee and Chaudhari (1983). All the observations were taken in triplicates and data were analysed statistically.

Experimental findings on antioxidant system indicate that two genotypes responded differentially to high temperature conditions. The superoxide dismutase activity enhanced continuously with increasing temperature; the magnitude was comparatively lower in HD 2329. It was further noted that the increment in the activity of this enzyme after 35ºC was significant only in C-306 (Table 1). It is well known that plants have a well organized defense system against reactive oxygen species and superoxide dismutase constitutes the first line of defense via detodification of superoxide radicals (sairam *et al*. 2000). Comparatively higher increment of superoxide dismutase activity in C-306 might have decreased the possible toxic concentration of O^-_2 radical more effectively. Mazorra *et al*. (2002) also reported the role of superoxide dismutase activity in imparting temperature stress tolerance. Peroxidase, another important H_2O_2 scavenging enzyme also followed the similar trend (Table 1). Increase in peroxidase activity under stress conditions have been linked with protection from oxidative damage, lignification and cross linking of cell wall to prevent from such adverse conditions (Dalal and Khanna-Chopra 2001). The decrease in catalase activity in HD 2329 could indicate its inactivation by the accumulated H_2O_2 induced by high temperature which might caused photoinactivation of this enzyme. It is also suggested that higher concentrations of catalase and ascorbate peroxidase might have also removed the heat stress induced by O^-_2 and its product H_2O_2 (Sairam *et al*. 2000). Nayyar and Kaushal (2002) also reported that the increased activity of catalase and peroxidase enzyme constitute potential defense mechanism against chilling induced oxidative damage in germinating wheat grains.

The MDA content was significantly higher in HD 2329 over C-306 under high temperature conditions. At room temperature, hydrogen peroxide was at par in both the genotypes but at 35ºC and 40ºC, susceptible genotype HD 2329 registered a linear and significant enhancement in H_2O_2 content (Table 1). It is known that H_2O_2 is a toxic compound which is produced as a result of scavenging of superoxide radicals. Its higher concentration is injurious to plants via lipid peroxidation and membrane injury (Nayar and Kaushal 2002). The lower values of MDA and H_2O_2 content in present investigation indicate that at cellular level C-306 is better equipped with an efficient free radical quenching system that offers protection against oxidative stress.

Relative water content decreased significantly in leaf discs of both the genotypes incubated at 35 and 40ºC. It was higher in HD 2329 under room temperature conditions but at higher temperatures C-306 retained higher water

Table 1. High temperature induced changes in antioxidants and lipid peroxidation in wheat seedlings.

Parameter	C-306			HD 2329			CD
	25ºC	35ºC	40ºC	25ºC	35ºC	40ºC	(0.05)
Superoxide dismutase activity (unit × 10^{-2} g^{-1} fw)	62.30	79.23	92.51	64.27	72.24	74.91	3.75
Peroxidase activity (Δ470 g^{-1} fw min^{-1})	44.00	54.88	66.28	36.15	42.31	45.05	3.21
Catalase activity (µmol H_2O_2 reduced g^{-1} fw)	4.18	7.15	9.54	2.72	6.53	7.15	1.07
Malondialdenhyde content (µmol g^{-1} fw)	3.69	4.08	4.11	3.98	5.12	5.29	0.29
Hydrogen peroxide content (µmol g^{-1} fw)	729	810	852	795	921	964	59.2

content (Fig. 1). It is suggested that the higher relative water content could help the tolerant genotype to perform physio-biochemical processes more efficiently under high temperature conditions. Membranes are the main loci affected by high temperature conditions. In present investigations, tolerant wheat genotype, C-306 maintained a higher membrane stability index at room as well as higher temperatures (Fig. 1). The lower membrane stability reflects the extent of lipid peroxidation, which in turn is a consequence of higher oxidative stress due to high temperature conditions. Our data on H_2O_2 content also support these findings (Table 1). Enhanced water retention and membrane stability in tolerant wheat genotypes have also been observed in other studies (Deshmukh *et al*. 1991).

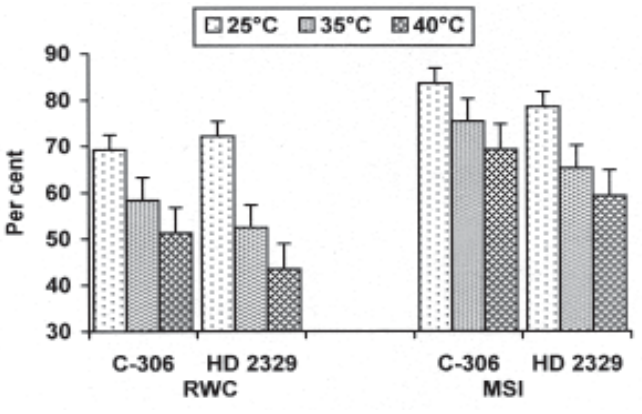

Fig. 1. Effect of temperature on relative water content (RWC) and membrane stability index (MSI) in wheat seedlings

On the basis of these results it can be inferred that the temperature tolerance mechanism exists at seedling stage of wheat genotypes. The C-306 is comparatively higher temperature tolerant owing to the lower increase in H_2O_2 and MDA content along with higher increase in superoxide dismutase, peroxidase and catalase activities. The higher membrane stability index and high water retention capacity might have also imparted temperature tolerance in C-306. Further, HD 2329 was also able to resist high temperature stress to some extent via above adjustments.

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