BIOLOGIA PLANTARUM 44 (1): 89-94, 2001

Comparison of hexaploid and tetraploid wheat cultivars in their responses to water stress

R.K. SAIRAM, V. CHANDRASEKHAR and G.C. SRIVASTAVA

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi-110 012, India

Abstract

We studied the effect of water stress imposed at anthesis and pre-anthesis stages on oxidative stress and antioxidant activity in four wheat cultivars, two hexaploid *Triticum aestivum* cultivars, drought resistant cv. C 306 and drought susceptible cv. Hira, and two tetraploid cultivars, *T. durum* cv. A 9-30-1 and *T. dicoccum* cv. HW 24. Water stress decreased relative water content (RWC), membrane stability index (MSI), and increased H_2O_2 and malondialdehyde (MDA) contents as well as activity of superoxide dismutase (SOD), catalase (Cat) and peroxidase (POX) in all the genotypes at all the stages. Both the tetraploid cultivars showed higher RWC, MSI and SOD activity, and lower H_2O_2 and MDA contents under water stress than hexaploid ones. Cat and POX activities were highest in C 306.

Additional key words: catalase, hydrogen peroxide, malondialdehyde, membrane stability index, peroxidase, ploidy level, superoxide dismutase, *Triticum aestivum*, *T. dicoccum*, *T. durum*, water stress.

Introduction

Shortage of water limits plant growth and crop productivity in arid regions more than any other environmental factor (Boyer 1982). The responses to drought have proved to be very complex, involving nearly every major function of plant growth (Kauffmann and Hall 1974). Due to their better adaptation under hot and arid regions tetraploid genotypes of wheat are usually regarded as more tolerant to stress condition than hexaploid wheat genotypes (Percival 1974, Waines 1994). However, studies explaining mechanism of their moisture stress tolerance are very few.

Percival (1974) reported that durum wheat requires a dry hot climate for satisfactory growth. Al-Hakimi and Mannoveux (1993) reported that tetraploid wheats, *e.g. T. dicoccum* accessions have high LWP, RWC, biomass production, number of tillers and spikes per plant, and *T. polonicum* (tetraploid) has high peduncle and awn lengths, good spike fertility, high kernel mass, and a good superficial rooting pattern under drought. Damania and Tahir (1993) have also reported high degree of heat tolerance due to AABB genome of durum wheat.

In recent years various studies have established that water, temperature and salinity stress induced generation of superoxide radical, hydrogen peroxide and hydroxyl radical, as the major cause of stress induced injury experienced at cellular and at crop levels (Elstner 1987, Baisak *et al.* 1994). Antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, peroxidase, and metabolites like ascorbic acid, glutathione, α-tocopherol, flavanoids, carotenoids, *etc.*, have been linked to stress tolerance in various crop plants (*e.g.* Liebler *et al.* 1986, Elstner 1987, Larson 1988). Increased activity of various antioxidant enzymes under water and temperature stresses have been reported by various workers (*e.g.* Matters and Scandalios 1986, Baisak *et al.* 1994). However, reports regarding variations in their activity on genotypic basis or due to ploidy levels are very few and inconsistent in their conclusions. Badiani *et al.* (1990) studied the relation between drought stress and enzymatic antioxidant systems

---

Received 5 April 2000, *accepted* 26 August 2000.

Abbreviations: Cat - catalase; MDA - malondialdehyde; MSI - membrane stability index; $O_2^{\cdot-}$ - superoxide radical; POX - peroxidase; RWC - relative water content; SOD - superoxide dismutase.

Acknowledgement: V. Chandrasekhar is grateful to Indian Council of Agricultural Research, New Delhi, for the award of Research Fellowship during the course of the study.

Fax: (+91) 011 5766420, e-mail: rks_ppl@yahoomail.com

in wheat species of different ploidy levels (*T. aestivum*, hexaploid; *T. persicum*, tetraploid; *T. monococcum*, diploid), though they did not compare and discuss their results in relation to ploidy levels. Zhang and Kirkham (1994) concluded that hexaploid wheat have less efficient antioxidant systems (other than SOD and catalase) as they found increased MDA content in the hexaploid wheat as compared to tetraploids and diploids.

As very little work has been reported on the role of cellular antioxidant systems in relation to stress tolerance in tetraploid wheat, an experiment was conducted with two tetraploid and two hexaploid wheat cultivars to investigate the significance of antioxidant systems in drought tolerance of tetraploid wheat.

Materials and methods

The present investigation was undertaken during the winter season of 1998 - 1999 with two hexaploid *Triticum aestivum* L. cultivars, drought resistant cv. C 306 and drought susceptible cv. Hira (HD 1945), and two tetraploid cultivars, *T. durum* cv. A 9-30-1 and *T. dicoccum* cv. HW 24. Seedlings were grown in earthen pots (30 × 30 cm) filled with clay-loam soil and farm yard manure (6: 1) under natural conditions. After germination 4 seedlings were retained in each pot. Fifty pots of each genotype were maintained in the net house. Plants were watered as and when required to keep them fully turgid. Recommended cultural practices were followed. Water stress was imposed by withholding water supply for 7 d during three different phases, at 50 % anthesis and 10 and 20 days after anthesis (DAA). Samples were collected from the flag leaf at 7, 17 and 27 DAA from control and stressed plants between 9.30 to 10.30. Soil moisture content (percentage of oven dried soil) varied between 29.85 to 30.56 % in control pots and from 11.12 to 12.38 % in non-irrigated pots.

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Weatherley (1950). Leaf membrane stability index (MSI) was determined according to the method of Premachandra *et al.* (1990) as modified by Sairam (1994). Small discs from the leaves were cut, weighed (0.2 g) and kept in 10 cm^3 of double distilled water at 40 °C for 30 min. After incubation, the electrical conductivity of the water containing the sample was measured (C_1) using conductivity bridge. Test tubes in second set were incubated at 100 °C for 15 min and their electrical conductivity measured as above (C_2) and MSI was calculated: $MSI = [1 - (C_1/C_2)] \times 100$.

Hydrogen peroxide was estimated according to Mukherjee and Choudhuri (1983). Leaf samples (0.5 g) were homogenized in 10 cm^3 of cold acetone and filtered through *Whatman No. 1* filter paper. To the extract 4 cm^3 of titanium reagent (Teranishi *et al.* 1974) was added followed by 5 cm^3 of concentrated ammonia solution to precipitate the peroxide-titanium complex. After centrifugation for 5 min at 10 000 *g*, the supernatant was discarded and precipitate was dissolved in 10 cm^3 of 1 M H_2SO_4 and absorbance was recorded at 415 nm against blank. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the method of Heath and Packer (1968). Leaf sample (0.5 g) homogenized in 10 cm^3 of 0.1 % trichloroacetic acid (TCA) was centrifuged at 15 000 *g* for 15 min. To 2 cm^3 aliquot of the supernatant 4.0 cm^3 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 an ice bath. The absorbance of the supernatant was recorded at 532 nm. The value for non specific absorption at 600 nm was subtracted. The MDA content was calculated using its absorption coefficient of 155 $mmol^{-1}$ cm^{-1}.

Enzyme extract was prepared by homogenizing leaf material (0.5 g) with 5 cm^3 of ice cold phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA, followed by centrifugation at 4 °C for 15 min at 15 000 *g* in a *Beckman* refrigerated centrifuge (Geneva, Switzerland) model *J2-21*. The supernatant was referred to as enzyme extract. Superoxide dismutase activity was estimated according to the method of Dhindsa *et al.* (1981). The 3 cm^3 reaction mixture consisted of 150 μmol sodium carbonate, 40 pmol methionine, 0.225 μmol nitroblue tetrazolium, 0.3 μmol EDTA, 150 μmol potassium phosphate buffer (pH 7.5), 1.0 cm^3 distilled water and 0.05 cm^3 of enzyme. The reaction was started by adding 6 nmol riboflavin and placing the tubes below two 15 W fluorescent lamps (irradiance of 80 μmol m^{-2} s^{-1}) for 15 min. Switching off the light and covering the tubes with black cloth stopped reaction. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture, which did not develop colour, served as blank. Absorbance was recorded at 560 nm in a *Beckman* (Geneva, Switzerland) mode*l M-36* UV-visible spectrophotometer, and one unit of enzyme activity was taken as that quantity of enzyme which reduced the absorbance reading of samples to 50 % in comparison with the samples lacking enzyme. Catalase was assayed according to Teranishi *et al.* (1974). The reaction mixture in 3-cm^3 cuvette consisted of 6 μmol H_2O_2 and 190 μmol potassium phosphate buffer (pH 7.0), and the reaction was initiated by adding 0.1 cm^3 of diluted (5 times) enzyme extract. The reaction was stopped after 5 min by the addition of 4 cm^3 of titanium reagent, which also forms coloured complex with residual H_2O_2. Reaction mixture without enzyme served as a control and developed maximum colour with titanium reagent. Aliquot was centrifuged at 10 000 *g* for 10 min and absorbance of the supernatant was recorded at 415 nm spectrophotometrically. Peroxidase assay was done according to the method of Castillo *et al.* (1984). It was performed at 25 °C in a 3.0-cm^3 cuvette containing 210 µmol potassium phosphate buffer (pH 6.1), 48 µmol guaiacol and 0.1 cm^3 enzyme extract (diluted 5 times). Reaction was initiated by adding 6 µmol H_2O_2 in the last. Increase in absorbance was recorded at 470 nm spectrophotometrically over a period of 2 min.

Ten pots of each genotype were subjected to water stress at 50 % anthesis for 7 d. After the expiry of stress period the pots were irrigated and kept separately for yield analysis. After maturing, the plants were harvested from stressed and regularly irrigated pots. The parameters like plant height and total plant biomass were obtained for comparative study on influence of moisture stress on such characteristics. Experiment was laid out in complete randomized design. All biochemical observations are means of four replications repeated twice. Data was analyzed by analysis of variance to determine the significance of treatments and cultivars.

Results

Tetraploid cultivars, A 9-30-1 followed by HW 24, retained higher RWC under water stress than the two hexaploid cultivars (Hira and C 306) (Fig. 1*A*) at all stages. Hydrogen peroxide content increased in all water stressed plants over the irrigated plants (Fig. 1*B*), and H_2O_2 accumulation under water stress was higher in hexaploid cultivars as compared to tetraploid cultivars at all the stages. Among the hexaploid cultivars C 306 accumulated less H_2O_2 than Hira.

Lipid peroxidation estimated as MDA content (Fig. 1*C*) increased under water stress and also with plant age in all the genotypes studied. Hira showed maximum MDA content followed by C 306, A 9-30-1 and HW 24 at the three stages of sampling.

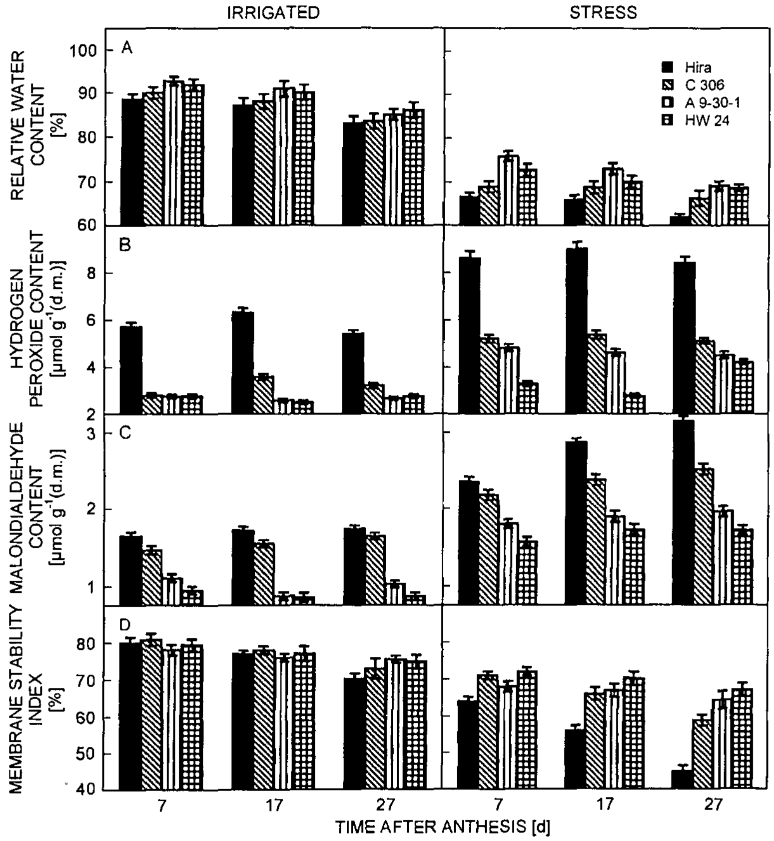

Fig. 1. Effect of water stress at different developmental stages on relative water content (*A*), hydrogen peroxide content (*B*), lipid peroxidation (*C*), and membrane stability index (*D*), in tetraploid and hexaploid wheat genotypes. *Vertical bars* represent SE of mean, *n* = 8.

Membrane stability index (MSI) decreased under water stress as well as with plant age in all the genotypes studied (Fig. 1*D*). More pronounced reduction in MSI under water stress was observed in drought susceptible cultivar Hira, followed by C 306, A 9-30-1 and HW 24.

Superoxide dismutase, catalase and peroxidase activities increased significantly under water stress in all the genotypes at all the three stages of study (Fig. 2). SOD activity both under irrigated and water stress conditions increased at second stage and then declined at third stage in Hira, C 306 and A 9-30-1, while in HW 24 in control plants activity did not decrease at third stage and was the highest under water stress condition. Tetraploid cultivars showed higher SOD activity as well as per cent increase under water stress over irrigated control as compared to the hexaploid cultivars. Catalase activity under water stress did not vary much among Hira, C 306 and HW 24, however, A 9-30-1 showed significantly lower activity at all the stages. Hexaploid cultivar C 306 followed by Hira showed consistently higher peroxidase activity than tetraploid cultivars A 9-30-1 and HW 24 at all the stages of sampling.

Hira, a triple dwarf had minimum plant height, but it showed maximum reduction in plant height, followed by C 306, A 9-30-1 and HW 24 in decreasing order (Table 1). Total dry mass per plant decreased under water stress in all the cultivars and was more pronounced in the hexaploid cultivars.

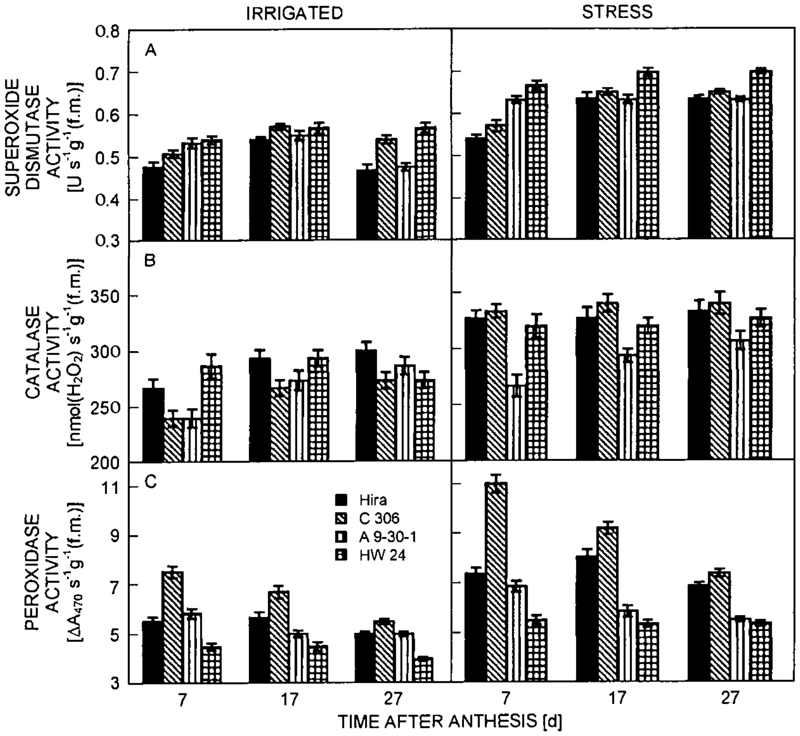

Fig. 2. Effect of water stress at different developmental stages on the activity of superoxide dismutase (*A*), catalase (*B*), and peroxidase (*C*) in tetraploid and hexaploid wheat genotypes. *Vertical bars* represent SE of mean, $n = 8$.

Table 1. Effect of water stress on plant height and dry mass production in hexaploid (C 306 and Hira) and tetraploid (A 9-30-1 and HW 24) wheat cultivars. Stress was imposed at 50 % anthesis and measurements were done at maturity.

Genotype	Plant height [cm]			Dry mass [g plant^{-1}]		
	irrigated	stress	decrease [%]	irrigated	stress	decrease [%]
Hira	62.00 ± 5.37	57.50 ± 3.27	6.85	47.75 ± 2.18	29.03 ± 3.28	39.20
C 306	85.50 ± 3.84	82.00 ± 4.51	4.09	46.16 ± 3.51	31.68 ± 3.51	31.30
A 9-30-1	92.00 ± 3.69	88.25 ± 3.85	4.08	47.33 ± 3.47	36.44 ± 2.89	23.00
HW 24	86.70 ± 4.51	83.75 ± 3.46	3.46	49.16 ± 3.29	39.83 ± 3.41	18.98

Discussion

Wheat crop responds to water deficit in the form of changes in various physiological and biochemical processes. Water stress imposed during anthesis and post anthesis showed that tetraploid wheat cultivars A 9-30-1 and HW 24 were able to retain higher RWC, membrane stability, and lower H_2O_2 and malondialdehyde content under water stress than hexaploid cultivars. The activity of antioxidant enzyme SOD was also higher in both tetraploid as compared with hexaploid cultivars. However, catalase and peroxidase activities (responsible for H_2O_2 scavenging) were higher in hexaploid C 306 (drought tolerant) and Hira (drought susceptible) compared to both the tetraploids.

Considering also the maintenance of higher total dry matter and plant height, it can be concluded that both tetraploid cultivars HW 24 and A 9-30-1 are more tolerant to water stress than the hexaploid cultivars C 306 and Hira. However, C 306 was not far behind and was superior to Hira. Better drought adaptability of tetraploids over hexaploid has also been reported by Percival (1974) and Hanson *et al.* (1982).

H_2O_2 is a toxic reactive oxygen compound and its contents are related to lipid peroxidation and membrane injury/stability (Pastori and Trippi 1992, 1993, Baisak *et al.* 1994, Sairam *et al.* 1998). Premachandra *et al.* (1990) have reported that cell membrane stability is an indicator of stress tolerance. Membrane stability/injury has also been related with the tolerance/susceptibility of various crop plants (Sairam *et al.* 1990, Sairam 1994, Kraus *et al.* 1995, Sairam 1997/98).

Superoxide dismutase is associated with the scavenging of superoxide radical ($O_2^{\cdot-}$) resulting in the formation of hydrogen peroxide (Fridovich 1986, Elstner 1987). The highest activity of SOD in HW 24 is consistent with lowest oxidative stress in terms of lipid peroxidation and highest membrane stability observed in it. Activity of H_2O_2 scavenging enzymes, catalase and peroxidase were, however, maximum in hexaploid drought resistant C 306, followed by Hira (susceptible to water stress) and lowest in tetraploid cultivars HW 24 and A 9-30-1. Zhang and Kirkham (1994) have observed that lower H_2O_2 accumulation and malondialdehyde content in wheat genotypes of lower ploidy level (diploids and tetraploids) were not associated with their antioxidant activity. Lower catalase and peroxidase activity in HW 24 and A 9-30-1 could also be a consequence of reported induction of their activity and/or synthesis of these enzyme proteins by H_2O_2 (Pastori and Trippi 1992, Prasad *et al.* 1994). As there was less accumulation of H_2O_2 in HW 24 and A-9-30-1, it might have resulted in decreased induction or activation of catalase and peroxidase enzymes.

It is also possible that other systems responsible for H_2O_2 scavenging may be more efficient in tetraploids, resulting in reduced tissue H_2O_2 content. One such system is ascorbate peroxidase-glutathione reductase cycle (Foyer and Halliwell 1976, Gillham and Dodge 1986, 1987, Nakano and Asada 1981, Asada 1992). A verv high activity of ascorbate peroxidase and glutathion reductase have been reported in tolerant genotype of wheat (Sairam *et al.* 1997/98, 1998). It can be hypothesized that since catalase and peroxidase are not efficient in HW 24 and A 9-30-1, the cause of lower H_2O_2 accumulation could be possible involvement of ascorbate peroxidase-glulathione reductase systems. However, this aspect needs to be investigated.

From the foregoing discussion it can be concluded that lower reduction in plant height and biomass under condition of water stress in tetraploid cultivars A 9-30-1 and HW 24 than hexaploid cultivars C 306 and Hira are possibly associated with their lower H_2O_2 accumulation, lipid peroxidation, higher membrane stability and activity of superoxide dimutase. C 306, a rainfed variety, though was slightly inferior to HW 24 and A-9-30-I, was superior to Hira (water stress susceptible). It is also apparent that superior drought tolerance of tetraploids over hexaploids is not limited to a particular physiological character, and all the parameters associated with drought stress tolerance may not be uniformly favourable in all the tolerant genotypes. As we have earlier reported that while tetraploid HW 24 and A 9-30-1 accumulated more ABA during water stress, they show lower proline accumulation than hexaploid cultivars C 306 and Hira under water stress (Chandrasekhar *et al.* 2000), though accumulation of both metabolites is associated with drought tolerance.

References

Al-Hakimi, A., Mannoveux, P.: Morphophysiological traits related to drought tolerance in primitive wheats. - In: Damania, A.B. (ed.): Biodiversity and Wheat Improvement. Pp. 199-217. Academic Press, New York 1993.

Asada, K.: Ascorbate peroxidase - a hydrogen peroxidase scavenging enzyme in plants. - Physiol. Plant. **85**: 235-241, 1992.

Badiani, M., De Biasi, M.G., Colognola, M., Artemi, F.: Catalase, peroxidase and superoxide dismutase activities in seedlings submitted to increasing water deficit. - Agrochimica **34**: 90-102, 1990.

Baisak, R., Rana, D., Acharya, P.B., Kar, M.: Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. - Plant Cell Physiol. **35**:

489-495, 1994.

Boyer, J.S.: Plant productivity and environment. - Science **218**: 443-448, 1982.

Castillo, F.L, Penel, L., Greppin, H.: Peroxidase release induced by ozone in *Sedum album* leaves. - Plant Physiol. **74**: 846-851, 1984.

Chandrasekhar, V., Sairam, R.K., Srivastava, G.C.: Physiological responses of hexaploid and tetraploid wheat to water stress. - J. Agron. Crop Sci., in press, 2000.

Damania, A.B., Tahir, M.: Heat and cold tolerance in wild relatives and primitive forms of wheat. - In: Damania, A.B. (ed.): Biodiversity and Wheat Improvement. Pp. 217 - 224. Academic Press, New York 1993.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. - J. exp. Bot **32**: 93-101, 1981.

Elstner, E.F.: Metabolism of activated oxygen species. - In: Davies, D.D. (ed.): The Biochemistry of Plants. Vol II. Pp. 253-315. Academic Press, San Diego 1987.

Foyer, C.H., Halliwell, B.: The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. - Planta **133**: 21-25, 1976.

Fridovich, L.: Biological effect of superoxide radical. - Arch. Biochem. Biophys. **247**: 1-11, 1986.

Gillham, D.J., Dodge, A.D.: Hydrogen peroxide-scavenging systems with in pea chloroplasts. A quantitative study. - Planta **167**: 246-457, 1986.

Gillham, D.J., Dodge, A.D.: Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: seasonal variations. - Plant Sci. **50**: 105-109, 1987.

Hanson, H., Borlaug, N.E., Anderson, R.G.: Wheat in the Third World. - Westview Press, Boulder 1982.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Kauffmann, M.R., Hall, A.E.: Plant water balance - its relationship to atmospheric and edaphic condition. - Agr. Meteorol. **14**: 85 - 98, 1974.

Kraus, T.E., Mc Kersie, B.D., Fletcher, R.A.: Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. - J. Plant Physiol. **145**: 570-576, 1995.

Larson, R.A. : The antioxidants of higher plants. - Phytochemistry **27**: 969-978, 1988.

Liebler, D.C., Kling, D.S., Reed, D.J.: Antioxidant protection of phospholopid bilayers by α-tocopherol. Control of α-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. - J. biol. Chem. **261**: 12114-12119, 1986.

Matters, G.L., Scandalios, J.G.: Effect of the free radical generating herbicide paraquat on the expression of superoxide dismutase (SOD) genes of maize. - Biochem. biophys. Acta **882**: 29-38, 1986.

Mukherjee, S.P., Choudhuri, M.A.: Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. - Physiol. Plant. **58**: 166-170, 1983.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Pastori, G.M., Trippi, V.S.: Oxidative stress induces high rate of glutathione reductase synthesis in a drought resistant maize strain. - Plant Cell Physiol. **33**: 957-961, 1992.

Pastori, G.M., Trippi, V.S.: Crop resistance between water and oxidative stresses in wheat leaves. - J. agr. Sci. **120**: 289-294, 1993.

Percival, J. (ed.): The Wheat Plant: A Monograph. - Redwood Burn Ltd., London 1974.

Prasad, T.K., Anderson, M.D., Martin, B.A., Steward, C.A.: Evidence for chilling induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. - Plant Cell. **6**: 65-74, 1994.

Premachandra, G.S., Saneoka, H., Ogata, S.: Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soyabean. - J. agr. Sci. **115**: 63-66, 1990.

Sairam, R.K.: Effect of moisture stress on physiological activities of two contrasting wheat genotypes. - Indian J. exp. Biol. **32**: 594-593, 1994.

Sairam, R.K., Deshmukh, P.S., Saxena, D.C.: Role of antioxidant systems in wheat genotypes tolerance to water stress. - Biol. Plant. **41**: 387-394, 1998.

Sairam, R.K., Deshmukh, P.S., Shukla, D.S., Ram, S.: Metabolic activity and grain yield under moisture stress in wheat genotypes. - Indian J. Plant Physiol. **33**: 226-231, 1990.

Sairam, R.K., Shukla, D.S., Saxena, D.S.: Stress induced injury and antioxidant enzymes in relation to drought tolerance in wheat genotypes. - Biol. Plant. **40**: 357-364, 1997/98.

Teranishi, Y., Tanako, A., Osumi, M., Fukui, S.: Catalase activity of hydrocarbon utilising *Candida* yeast. - Agr. Biol. Chem. **38**: 1213-1216, 1974.

Waines, J.G.: High temperature stress in wild wheats and spring wheats. - Aust. J. Plant Physiol. **21**: 705-715, 1994.

Weatherley, P.E.: Studies in the water relations of cotton plants. I. The field measurement of water deficit in leaves. - New Phytol. **49**: 81-87, 1950.

Zhang, J., Kirkham, M.B.: Drought stress induced changes in activities of superoxide dismutase, catalase and peroxidase in wheat species. - Plant Cell Physiol. **35**: 785-791, 1994.