# Antioxidant Defense System and Lipid Peroxidation in Relation to Drought Tolerance in Wheat (Triticum aestivum L.)

# THESIS

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**S.K.N. COLLEGE OF AGRICULTURE, JOBNER**

## CERTIFICATE - I

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This is to certify that **Ms. Sunita Gupta** had successfully completed the preliminary/comprehensive examination held on 26-10 2002 as required under the regulation for **DOCTOR OF PHILOSOPHY** in Agriculture.

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This is to certify that this thesis entitled **"Antioxidant Defense System and Lipid Peroxidation in Relation to Drought Tolerance in Wheat (*Triticum aestivum* L.)"** submitted for the degree of **DOCTOR OF PHILOSOPHY** in **Agriculture** in the subject of **Plant Physiology** is a bonafide research work carried out by **Ms. Sunita Gupta** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 12-12-2003.

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*Chapter 1.*

# INTRODUCTION

The extent to crops perform in terms of their biological/economic yield is decided to a large extent by the interactions of their genetic potential with the environment. In the complex field-level situation, the environmental factors generally exert both growth promoting as well as growth retarding effects. The relative balance of these counter-active effects determine whether the crop yield optimally or not. Different plant processes (starting with seed germination, emergence and growth of the seedlings, production of vegetative organs, maintenance of various metabolic activities including the function of light absorption and carbon assimilation, onset and intensity of flowering, extent of fruit set to the eventual course of senescence) are affected differentially by a large number of biotic and abiotic stresses (Nilsen and Orcutt 1996, Aducci 1997, Galum and Breiman 1997, Lerner 1999). Drought is one of the abiotic factor affecting physiological processes of the plant and by far the most important and dominant factor which adversely affect crop production in the present wold (Kramer and Boyer 1995). It affects practically every aspects of plant growth by modifying various morpho-physiological and biochemical processes of plants (Singh and Singh 1994). Consequently, considerable emphasis has been given to the problem of drought tolerance.

Much of the injury to plants caused by stress exposure is associated with oxidative damage at cellular level (Allen 1995). Photosynthesizing aerobic organisms are regularly subjected to varying degrees of oxygen toxicity during their life cycles. However, in higher plants this oxygen toxicity is more serious under conditions of water deficit, resulting in a drastic decline in the levels of $CO_2$, NADP and increased transfer of electrons to oxygen, leading to the formation of the superoxide radical ($O_2^-$ ). The superoxide radical and its dismutation product, hydrogen peroxide, can directly

attack membrane lipids and inactivate SH-containing enzymes (Navari-Izzo *et al*. 1994, Sairam and Srivastava 2001). In the presence of trace amounts of iron salts the combination of $O_2^-$ and $H_2O_2$ leads rapidly to the formation of the hydroxyl radical ($OH^-$) (Halliwell, 1987). 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 (Imlay and Linn 1988, Becana *et al*. 1998). Plants protect cell and sub cellular systems 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 (Larson 1988, Alscher *et al*. 2002).

The membranes are reported to damage rapidly with increasing water stress (Sgherri and Navari-Izzo 1995). This leakiness of membranes is caused by an uncontrolled increase in free radicals which cause lipid peroxidation (Smirnoff 1993). Impairment of the electron transport chain in chloroplast has been observed due to drought stress, which results in leakage of electrons and the subsequent formation of reactive oxygen species. The stress induced burst in free radicals could also be partly related to the activity of lipoxygenases, which convert C18:2 and C18:3 to the corresponding hydroxyperoxides (Bell and Mullet 1991). Further, damage to fatty acid could then produce small hydrocarbon fragments including malondialdehyde (MDA) (Kappus 1985, Aziz and Larcher 1998, Alscher *et al*. 2002).

An increased activity of protective enzymes such as superoxide dismutase, peroxidase and catalase may be a part of general antioxidative system in plants involving regulation of protein synthesis or gene expression (Bowler *et al.* 1992, Foyer *et al*. 1994, Rebetzke *et al*. 2003). Superoxide dismutase is an inducible enzyme (Hassan and Scandalion 1990); a high level of superoxide dismutase activity is associated with low level of lipid peroxidation during drought conditions and is also connected to drought tolerance (Bowler *et al.* 1992). Breakdown of $H_2O_2$ to water in the chloroplast is associated with ascorbic peroxidase and catalase (Asada 1992). Increased activity of various antioxidant enzyme under various stresses have

been reported by various workers (Matters and Scandalion 1986, Baisak *et al.* 1994). However, information on the antioxidant defense mechanism and its dynamics in tolerant and sensitive genotypes is quite lacking (Arora *et al*. 2002). Simultaneously, it has long been realized that identification, inducement and increase in drought and salinity tolerance can be investigated in contrasting genotypes, breeding for tolerance and using external application of bioregulants in crop plant.

Wheat, in addition to the staple food cereal of the world and country, provides the facility of genotypes contrasting in agronomic characteristics like drought and salt tolerance. This facility has led to investigation on collaborative research with physiological and breeding approaches for identifying rapid diagnostic traits (Chhipa *et al*. 1993) and also for analyzing the effect of plant growth regulators on stress tolerant attributes.

Plant growth regulators are potential tools to enhance crop production through redirecting the metabolism balance of growth and partitioning of assimilate which result in increase in quantity as well as quality of desired economic product (Nickel 1982, Nowak and Lawson 1983). Polyamines are a new group of polycationic low molecular weight nitrogenous compounds involved in several plant growth and developmental processes. Increased polyamines level in stressed plants are of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss and stimulation of synthesis of protein, nucleic acid and protective alkaloids (Sharma 1999).

The presence of polyamine has been detected in apoplast, plasma membrane, vacuole, cytosol, chloroplast and nuclei (Pistocchi *et al.* 1990). Cotreatment with polyamines reduces membrane leakage, lipid peroxidation (Tiburcio *et al.* 1994), senescence induced protease and ribonuclease activity (Kaur-Sawhney 1982, Sen and Ghosh 1984) and chlorophyll loss (Altman 1982). Yardanov and Golstev (1990) described a protective effect of putrescine and spermidine on the thylakoid membrane after high temperature treatments. Thus it seems that polyamines have an important

and innate role in combating stress effect but little is known on modulation of antioxidant enzyme activity in relation to drought tolerance.

Putrescine is one of the common polyamines present in plants. Generally massive accumulation of putrescine due to increase in arginine decarboxylase activity was observed in plant cells under abiotic stress conditions. The putrescine accumulation could be one of the adaptive mechanisms in plants to cope with various stresses conditions. Sharma (2001) reviewed the role of polyamines in agricultural and horticultural crops.

Polyamines have been postulated in the wide range of biological processes including cell division, growth and development (Golstev and Kaur-Sawhney 1995, Chattopadhyaya *et al*. 2002). Effect of plant growth regulators in enhancing growth and productivity of wheat has been observed under water stress and non stress conditions (Warrier *et al*. 1987, Gupta *et al*. 2000, 2003b). Currently polyamines have been viewed as an important group of chemical software for modulating crop responses under water limiting conditions. Effect of exogenous polyamines on olive (Rugini and Mencuccini 1985), rice (Yang et al. 1996) and soybean (Sharma and Ali 1998) have alraeady been reported. In our previous investigation polyamines were found to enhance productivity in wheat under non stress and water stress conditions in pot culture (Gupta et al. 2003). Despite much information available on the role of endogenous plant hormones and application of PGRs as seed treatment and crop spray, the much headway could not be made to translate the experimental information into a tenzible blue print of technology.

Sankhla et al. (1988) and Sharma (1999) indicate well that the interest in polyamines was predominantly on the metabolic aspects while their potential has modulation of growth and development was confined to the horticultural crop specially fruit plants. The regulation of oxidative metabolism, level of antioxidative defence agents and in nutshell, on the cellular characteristics of water stress tolerance is quite inadequate. Thus, looking to the imporatnce of wheat as food cereal, the availability of wheat genotypes contrasting in drought tolerance, the importance of cellular defense mechanism and potential of putrescine as a economically viable bioregulant, the investigation on "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)" has been proposed with described objectives:

1. To study variation in antioxidant enzyme activity and lipid peroxidation in wheat genotypes.

2. To study variation in antioxidant enzyme activity in relation to putrescine induced drought tolerance in wheat.
3. To evaluate the field efficacy of putrescine application.

# *Chapter 2.*

# Review of Literature

Stress tolerance has long been fascinated plant biologists as a challenge to understand the mechanism of plant survival under harsh environmental conditions like desert and also for the possibility of enhancing the productivity under sub-optimal and water limiting conditions (Levitt 1980). The research endeavours from eco-physiological standpoint have been dynamic and diversified on time space scale. The complexity in research efforts on drought tolerance was introduced mainly because of dichotomy of survival and productivity, adaptation, acclimation, inducement, enhancement and the levels of investigation like community, canopy, and organ, cellular and molecular (Morgan 1984, Grover *et al.* 1998).

Among conceptual transitions innate to plant biology abiotic stress tolerance, the cellular and enzymatic defense mechanism occupied center stage elbowing side the consideration at organ and canopy level. Down the traces of history a great surge of interest has been evident in the recent past about physiological responses of crop plants to water stress and mechanism of stress tolerance (Kozlowski 1968-1981, Paleg and Aspinall 1981, Christiansen and Lewis 1982, Boyer 1985, Yadava *et al*. 1994, Gupta *et al.* 2001, Grover and Chundramouli 2002). In addition, there are several review articles that scan the state of science in this area (Hsiao 1973,

Hellebust 1976, Turner and Jones 1980, Tanner and Sinclair 1983, Turner *et al.* 1986, Sinha *et al.* 1986, Srivastava and Kumar 1994, Arora *et al*. 2002, Rai 2002).

Wheat crop experiences water stress during its growth, when grown on conserved soil moisture and without irrigation. Therefore, the adaptability of this crop to variable water environments, including drought condition is an important factor in determining the productivity (Singh *et al.* 1985.). To this end, considerable efforts have been made in identifying physiological and morphological characters that are important for achieving stable yield under conditions of water stress. Several workers emphasized the need of breeding for various weather probabilities including temperature and precipitation (Boyer 1996, Rebetzke *et al.* 2003, Garg 2002). However, success in such breeding programmes is limited due to poor understanding of physiological traits associated with drought resistance. Baalbaki *et al*. (1999) reported that tolerance to drought could not be ascribed to one or two traits but was due to the ability of plants to develop a high number of tolerance mechanisms in terms of antioxidant defense system, growth, water relation parameters, photosynthetic activity, membrane stability, accumulation of metabolites, plant growth regulators etc.

The foregoing reviews of literature represent a selection of work undertaken national and international laboratories with particular emphasis on the cellular defense mechanism and related physiology of drought tolerance.

## 2.1 Antioxidant enzyme system

Closure of stomata under water stress is a major temporary adaptive change that prevents further water loss from the plants, which also lowers the influx of $CO_2$, resulting in the reduction of net photosynthetic capacity. However, photosynthetic electron transport is maintained at a relatively higher rate in the stressed leaves as compared to the large decrease in the rate of $CO_2$ fixation. This imbalance results in the accumulation of terminal electron acceptor, viz. NADPH and a decrease in NADP. Under such conditions, oxygen acts as an alternate electron acceptor, resulting

in the formation of superoxide radical ($O_2^-$) (Egneus *et al.* 1975, Allen 1995). It is hypothesized that a mechanism adapted by plants to protect the photosynthetic electron transport chain components from photo damage during water stress (Stuhlfauth *et al*. 1990). Superoxide radical and its reduction product $H_2O_2$ are potentially toxic compounds and may also combine by Habber Weiss reaction to form highly toxic hydroxyl radical ($OH^-$) (Elstner 1987). Active oxygen species, such as superoxide radical, hydrogen peroxide and hydroxyl radical can cause lipid peroxidation, resulting in the membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Fridovich 1986, Leibler *et al.* 1986, Imlay and Linn 1988). The detoxification of superoxide radical and hydrogen peroxide consequently is of prime importance in any defense mechanism. Plant protect cellular and subcellular system from the cytotoxic effect of these active oxygen radicals using enzymes such as superoxide dismutase, ascorbate peroxidase, peroxidase, glutathione reductase, catalase and metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Leibler *et al*. 1986, Elstner 1987, Larson1988). In different species, tissues and developmental stages tolerant to water stress, reduced membrane damage has been linked to the increased enzymatic defense against oxygen radicals, together with synthesis of free radical scavengers (Smirnoff 1993, Zhang and Kirkham 1994).

Describing the mode of action of these enzymes, Bowler *et al.* (1992) reported that superoxide dismutases react with superoxide radicals at almost diffusion-limited rates to produce hydrogen peroxide, which is disposed off by catalase and peroxidases. The peroxidases with broad specificities are often found in the cell wall, where they utilize $H_2O_2$ to generate phenoxy compounds, which then polymerize to produce cell wall components such as lignin (Greppin *et al*.1986). Glutathione reductase, the other key component, has a regulatory function because of the dependence of its activity on the availability of NADPH (Arora *et al.* 2002).

The role of plant antioxidant system in water stress tolerance was studied in contrasting wheat genotypes. Sairam *et al.* (1998) and Sairam and Saxena (2000) reported that water stress imposed at different stages after anthesis resulted in an

increase in lipid peroxidation and a decrease in membrane stability, chlorophyll and carotenoid content. The antioxidant enzyme ascorbate peroxidase, glutathione reductase and non-specific peroxidase increased significantly under water stress. Genotype C-306 and PBW 75 have the highest peroxidase, glutathione reductase and ascorbate peroxidase and highest membrane stability and content of chlorophyll and carotenoid under water stress, while the susceptible genotypes (HD 2329 and WH 542) exhibited lowest antioxidant enzyme activity, membrane stability, contents of chlorophyll, carotenoid and highest lipid peroxidation. It has been inferred that the drought tolerance of C-306 and PBW 175 is related to their higher antioxidant enzyme activity (Sairam *et al.* 1998). The study conduced by Bartoli *et al.* (1999) to evaluate oxidative stress and anti-oxidant response system in leaves of wheat (cv. Buck Poncho) subjected to drought and watering indicated that microsomes isolated from leaves exposed to drought followed by watering generated significantly higher amount of hydroxyl radicals as compared to microsomes isolated from control leaves. This suggests a higher reduction of hydroxyl radical in the cellular water-soluble phase after drought and watering as compared to control values. These active species, which are formed at wheat membrane after exposure to moderate water stress, are efficiently removed up on rehydration by reaction with an increased content of tocopherol and B-carotene. Moreover, a coordination activity, thiols, and ascorbic acid is triggered to limit free radical dependent effects. Differences in activities of SOD and CAT were related to a lower level of lipid peroxidation of cytoplasmic membranes and a longer life span in functional leaves during grain filling (Bartoli *et al.* 1999).

Baisak *et al.* (1994) confirmed that the increase in SOD activity could be decomposed in the chloroplast by the enzymes of the ascorbate glutathione pathway. Ascorbate peroxidase (APOX), the first enzyme of this pathway, increased at lower level of water stress when compared with control (Asada and Takahashi 1987, Salin 1988).

Similar to SOD activities, catalase activities increased at early stage of drought and declined in case of prolonged water stress (Zhang and Kirkham 1994).

They further reported that peroxidase activity increased greatly with water stress. Oswald *et al*. (1992) suggested that $H_2O_2$ scavenging system like catalase and glutathione reductase were more important in imparting tolerance against oxidative stress than SOD activity alone. Enhancing the $H_2O_2$ decomposing capacity of the leaves can be a strategy for imparting drought resistance in plants as has been suggested by Bowler *et al*. (1992).

Inci *et al*. (1999) and Sairam *et al*. (1998) reported that drought stress increased the activities of antioxidant enzymes like SOD, ascorbate peroxidase and CAT have significantly been accompanied with lower hydrogen peroxide accumulation and lipid peroxidation in the tolerant and adaptive cultivars of wheat while causing a decrease in enzyme activity and highest hydrogen peroxide content and lipid peroxation in sensitive cultivars. Burke *et al*. (1985) reported that glutathione reductase activity increased in drought stressed plants and it was proposed that in addition to removing $H_2O_2$, this increase might make NADP available that can accept electrons from ferredoxin thereby minimizing chances of superoxide formation. Importance of ascorbic acid and acsorbate peroxidase in amelioration of oxidative stress has also been reported (Gillham and Dodge 1987, Pastori and Trippi 1992, Baisak *et al*. 1994, Menconi *et al.* 1995). Catalase is also associated with scavenging of $H_2O_2$ and an increase in its activity is related with increase in stress tolerance (Upadhayaya *et al*. 1990, Olmos *et al*. 1994, Kraus 1995). A regulatory role of catalase in $H_2O_2$ scavenging and chilling resistance has also been reported by Prasad *et al*. (1994).

## 2.2 Hydrogen peroxide and lipid peroxidation

Photosynthetic plant cells are especially at risk from oxidative damage, because of their oxygenic conditions, the abundance of photosensitizers, polyunsaturated fatty acids in the chloroplast envelope and thylakoids (Halliwell 1987). Peroxidation is widely thought to be responsible for membrane deterioration during desiccation (Livne *et al.* 1990). Active oxygen species are known to be the main mediators of peroxidative components (Elstner 1987, Smirnoff 1993). The

harmful effect of these toxic radicals are, primarily due to their ability to initiate a variety of autoxidative chain reactions on unsaturated fatty acids, producing a carbon centers radical and lipid hydroperoxide that leads to lipid peroxidation and membrane destruction (Aziz and Larcher 1998). The superoxide radical, hydrogen peroxide radical ,hydroxyl radical and singlet oxygen are the four major activated oxygen species generated in plants tissues. Hydrogen peroxide and hydroxyl radicals are more active, toxic and destructive than the superoxide radical. Hydrogen peroxide can inactivate various Calvin cycle enzymes and is involved in metal catalysed oxidation systems, causing protein degradation (Asada and Takahashi 1987).

Lipid proxidation, an indicator of the prevalence of free radical reaction in the tissue, was determined in the water stressed leaves by estimating malondialdehyde content (a decomposition product of lipid peroxidation) as the thio-barbituric acid reactive material. Baisak *et al.* (1994) reported significant increase in malondialdehyde content in water stressed wheat leaves as compared to the control. They further concluded that in spite of enhanced activities of superoxide dismutase and glutathione reductase, the scavenging system against active oxygen species, specifically for $H_2O_2$ become less effective under higher degree of water stress. Mukherjee and Choudhary (1983) reported increase in the levels of $H_2O_2$ in *Vigna catajang* seedlings, subjected to higher degree of water stress but not to lower degree of water stress.

Baisak *et al.* (1994) also noticed the enhancement of lipid peroxidation in the leaves subjected to higher degree of water stress but not subjected to mild stress. The occurrence of lipid peroxidation is an indicator of the prevalence of free radical reactions in leaves. Further, they suggested that the changes in the balance between $O_2/H_2O_2$ might facilitate the metal-catalyzed formation of highly reactive hydroxyl radical through the Haber-weiss reaction, which may then initiate lipid peroxidation under severe water stress conditions. The decline in the $H_2O_2$ decomposing capacity of leaves is probably responsible for the occurrence of the oxidative damage and lipid peroxidation in water stressed leaves.

Dhindsa *et al.* (1981) reported that decrease in membrane stability reflects the extent of lipid peroxidation caused by active oxygen species. Lower lipid peroxidation and higher relative water content and membrane stability has also been reported in tolerant genotypes of maize (Pastori and Trippi 1992) and wheat (Kraus *et al*. 1995). Pastori and Trippi (1993) further confirmed that drought resistant wheat cultivars (Cruz Alta) showed lesser level of lipid peroxidation, when compared with drought sensitive ones (Leones). Zhang and Kirkham (1996) reported 33.7 per cent increase in MDA content (lipid peroxidation) of sunflower leaves when subjected to drought. Sairam *et al.* (1998) showed that drought tolerant wheat genotype (C-306) had lesser $H_2O_2$ content, a potent oxidant, and lipid peroxidation, while the drought susceptible genotype (HD 2329) and higher hydrogen peroxide content and lipid peroxidation (MDA content), which resulted in greater membrane injury.

## 2.3 Plant growth and development

Wet et al (1999) reported that drought inhibits the growth of coleoptiles in wheat, but the degree of inhibition of susceptible varieties was greater than resistant varieties. Under water stress growth rate of coleoptiles decreased resulting in shortening of coleoptiles length, the decrease of growth rate of resistant variety was less than susceptible variety. The capacity for coleoptile growth was highly correlated with osmoregulation ability. Baalbaki *et al*. (1999) also reported that drought resistant bread wheat cultivars had higher germination percentage and germination speed under moisture stress than susceptible cultivars, but the germination speed was more sensitive to change in osmotic potential than germination percentage. Reduction in growth under water stress condition has been reported in advanced developmental stages of growth. Gupta *et al.* (2001) reported that water stress imposed at boot and anthesis stages drastically reduced the plant height, leaf area, number of leaves and productive tillers. The effect was more pronounced in drought sensitive wheat genotypes.

## 2.4 yield and yield attributes

Weber and Hrynczuk (1999) reported that the sensitivity of cultivars to moisture stress was determined on the basis of the effect on yield components (plant height, productive tillering, ear length, grain number and grain weight). The difference observed in yield components among cultivars showed that the tested genotypes differed in drought defense mechanism. El *et al.* (1998) reported that lower relative growth rate longer grain filling period, increased number of kernel per spike and limited spikes per meter square can be used as selection criteria under water limited environment for drought resistance in durum wheat. The better drought tolerance of triticale is associated with an early heading date and greater capacity of root system to extract soil water (Giunta *et al.* 1993). Reduced canopy development in wheat before anthesis saves water loss, hence, leaf area index and high tiller survival rate are the morphological traits associated with higher yield of drought tolerant wheat cultivars (El *et al.* 1998).

The effect of water stress at anthesis stage of wheat was more detrimental than at tillering, fruiting and grain filling stages (Sairam *et al*. 1990, Kumar *et al.* 1993, Ravichandran and Munsge 1997, Gupta *et al.* 2001). Seed yield of wheat cultivars were 20-36, 61-70 and 28-39 per cent lower with water stressed and emergence to booting, booting to flower initiation and flower initiation to maturity stages, respectively compared with non stressed (Cracium *et al*. 1997). Drought in the period between stem elongation and heading seriously affected the leaf area index, radiation efficiency and biomass production of durum wheat (Giunta *et al.* 1995). It has also been reported that early drought limited primarily grain number per unit area while the late drought affected on grain weight (Ozturk 1999). The negative effect of early drought on grain yield was more significant than late drought. The reduction in grain yield of wheat cultivars under water stress is due to its effects on the different growth and yield attributes as reported by various authors. Waters stress decreased number of grains per spike (Wang *et al*. 1993), spike length (Jamal *et al*. 1996), number of spikes (Jat *et al.* 1991), tiller number per plant (Saha and Patel 1997, Weber and Hrynczuk 1999), shoot dry matter production (Hegazi *et al*. 1999) and leaf area (Jat *et*

*al*. 1990). Gupta *et al.* (2003a,b) suggested that reduction in grain yield under water stress was mainly on account of reduction in test weight.

Water stress also decreases harvest index and therefore, harvest index can be used as criteria to assess tolerance of wheat to drought (Stankova 1998). The correlation of soil drought tolerance with harvest index increased with time of exposure to drought. Correlation coefficient among yield and physiological traits of seven wheat genotypes grown in pots showed that leaf photosynthesis, total dry matter production, specific leaf weight, absolute growth rate, relative growth rate, net assimilation rate and harvest index were positively correlated with grain yield (Kumar *et al*. 1988). Gupta *et al.* (2000, 2001) also reported a significant correlation of water relation parameters with yield in drought tolerant and susceptible wheat genotypes at boot and anthesis stages.

## 2.4 Photosynthesis

Drought stress is one of the most important environmental factors inhibiting photosynthesis (Bradford and Hsiao 1982). The $CO_2$ fixation influences not only by the changed activity of the photosynthetic apparatus but also by the state of stomata which are dependent upon availability of water. Reduction in photosynthetic rate in wheat crop on account of water stress has been reported (Li *et al.* 1992, Janacek 1997, Iqbal and Wright 1998, Reddy 2000, Shangguan *et al.* 2000). However, the level of reduction in photosynthetic rate in drought tolerant varieties is less as compared to susceptible ones (Kumar *et al*. 1994, Sairam *et al*. 1990, Gupta *et al.* 2003a,b). Rekika *et al*. (1998) reported highest osmotic adjustment capacity of tolerant wheat cultivars under mild water stress condition. This osmotic adjustment allows for maintenance of photosynthesis by stomatal adjustment at low water potential (Zhouping *et al*. 1999). Ludlow (1980) indicated that large proportion of drought avoiding genotypes of wheat do not close stomata possibly because of osmotic adjustment of guard cells under water stress. Erichidi *et al*. (2000) found a positive and significant correlation between stomatal conductance and grain yield on durum wheat cultivars under water stress which indicated that the ability of cultivars to keep the stomata open may

constituted a drought resistance mechanism. Rekika *et al*. (1998) further reported that the decrease in photosynthetic rate under mild and severe water stress is mainly due to stomatal and non stomatal factors, respectively. Xue *et al*. (1992) observed half reduction in photosynthetic rate under mild water stress in susceptible and lesser reduction in tolerant wheat cultivars. Under severe water stress, in addition to the significant decrease in net photosynthesis, RUBISCO activity in leaves also decreased. This enzyme activity is affected earlier and to a greater extent in susceptible than tolerant cultivars.

Wang *et al*. (1992) reported that water stress decreased net photosynthesis in leaves. Stress caused increased activity of leaf glycolate oxidase and decreased RUBISCO activity. Stress disrupted the thylakoid lamellae, damaged cell membranes and starch grain disappeared while the number of lipid droplets in the thylakoid increased. El *et al.* (1998) observed that decrease in photosynthesis soon after imposition of drought stress resulted mainly from reduced stomatal conductance. Continued water deficit also reduced mesophyll photosynthetic activity. Further, the possible factors determining the drought resistance of a cultivar are lower sensitivity of $CO_2$ exchange rate, net $CO_2$ uptake to water loss ratio, stomatal resistance, relative water content and greater osmotic adjustment under stress.

Yin *et al.* (1995) reported large differences in photosynthetic rate, stomatal conductance to $CO_2$, stomatal resistance and intercellular $CO_2$ concentration in wheat cultivars. In general, the rate of photosynthesis and stomatal resistance were higher before anthesis than after anthesis but stomatal conductance to $CO_2$ and intercellular $CO_2$ were lower before anthesis. it was also observed that before anthesis stomatal factors were the main limitations in rate of photosynthesis but after anthesis other factors were limiting. Rebetzke *et al.* (2003) reported the expression of specific genes action for leaf conductance and stomatal regulation in tolerant wheat genotypes. The differential photosynthetic characteristics of flag leaf in wheat under adverse conditions has been observed by Mohanty (2003).

## 2.5 Plant water relation parameters

A significant reduction in leaf water potential and relative water content of wheat cultivars has been reported under water stress condition (Jat *et al*.1991, Singh *et al*. 1993), however, tolerant varieties showed lesser reduction as compared with susceptible varieties (Sairam *et al.* 1990, Boyer 1996). McCaig and Ramgosa (1989) in their study of excised leaf in tetraploid wheats reported that those adapted to dry land conditions exhibited high initial water content and low rate of water loss after leaf excision. Gupta *et al*. (2001) reported the correlation coefficient of grain and biological yield with water potential and its components was positive and highly significant. Similarly turgor potential was also correlated positively and significantly with grain yield.

Singh *et al.* (1993) reported significant parabolic correlation between leaf water potential and canopy temperature, linear reciprocal relations between leaf water potential and stomatal resistance and a linear correlation between stomatal resistance and canopy temperature. Decrease in transpiration rate (Singh *et al*. 1992), increase in leaf diffusive resistance (Kumar and Gour 1992, Strauss and Agenbag 2000) and increase in leaf temperature (Wang *et al*. 1993, Ravichandran and Munsge 1999) are the measures of drought tolerance in wheat cultivars. Rashid *et al*. (1999) reported significant correlation between canopy temperature and yield under water stress conditions in wheat cultivars. Gupta *et al.* (2001) reported a negative and significant correlation for diffusive resistance, leaf to air temperature gradient with grain yield and positive and significant correlation of transpiration rate and grain yield in drought tolerant and sensitive cultivars of wheat.

## 2.6 Osmolytes

Under water stress there was an increase in the content of some metabolites such as proline (Singh and Patel 1996, Saha and Paul 1997), amino acids (Prasad *et al.* 1999, Rai 2002) and sugars (Khan *et al.* 1999, Gupta *et al.* 2001). Khan *et al*. (1999) reported that occurrence of osmotic adjustment by accumulation of metabolites and thus maintains turgor by decreasing osmotic potential at low leaf water potential are the mechanism for drought tolerance in wheat genotypes. Blum *et*

*al*. (1999) concluded that genetic differences in osmotic adjustment (OA) existed among wheat cultivars and that high (OA) cultivars tended to yield better than low OA cultivars under pre flowering drought stress.

It is a well established fact that endogenous ABA levels increases considerably, when plants are subjected to water stress (Davies and Mansfield 1983). The increase in ABA inhibits growth and this adaptive plant response conserves moisture loss during prolonged drought (Quarrie and Jones 1977). Because ABA concentrations are governed primarily by plant water status, measurement of ABA may be a more stable indication of stomatal functioning during water stress than direct measurements of stomatal conductance itself. It may be a useful metabolite indicator of long term responses to drought (Quarrie and Jones 1977).

Osmotic adjustment has no effect on water use efficiency but it contributes to grain yield in water limited condition by increasing the amount of water transpired and by minimizing the reduction in harvest index (Morgan and Condon 1986, Santameria *et al*.1990). Increase in water transpired result from stomatal adjustment, maintenance of leaf area and increased soil water uptake. Osmotic adjustment reduces the rate of leaf senescence, because it increases both avoidance and tolerance of dehydration (Blum *et al.* 1999) .The osmotic adjustment (OA) has been found positively correlated with biomass and grain yield. It is a typically inducible trait, which could constitute a partial explanation of the association noted by several authors between OA capacity and yield stability (Morgan 1983, Morgan and Condon 1986). Studies carried out in our lab also revealed that tolerant wheat genotypes are able to perform better by osmoregulation (Jat *et al.* 1991, Singh *et al*. 1994).

## 2.7 Cell membrane stability

The degree of cell membrane stability under stress, evaluated by ion leakage, correlates well with tolerance of plant process. This technique has been used for identifying stress tolerant genotypes of sorghum (Sullivan and Ross 1979), wheat (Blum and Ebercon 1981, Gupta *et al*. 2000, Bajje *et al.* 2000), chickpea (Deshmukh and Kushwah 2002) and soybean (Premchandra *et al.* 1990). Deshmukh *et al*. (1991)

have studied the possibility of using ion leakage as simple index for grouping of genotypes, according to their tolerance capacity to moisture in wheat. Singh *et al*. (1992) and Sairam *et al*. (1998) observed higher membrane stability in drought and temperature tolerant wheat genotypes under stress conditions. Higher cell membrane stability in drought tolerant wheat genotypes under water stress is due to increased activities of antioxidative enzymes which prevents damage of membrane by active oxygen species produced under stress environment (Sairam and Saxena 2000). Use of exogenous application of cytokinins also found to increase the membrane stability index in wheat (Gupta *et al*. 2000).

## 2.8 Chlorophyll and carotenoid contents

The reduction in the contents of chlorophyll and carotenoids in plants, subjected to water stress has widely been demonstrated. Baisak *et al.* (1994) reported the decline in chlorophyll content with water stress. Sgherri and Navari-Izzo (1995) reported the decrease in the levels of carotenoids and hydrophobic proteins in the thylakoids and increase in lipids:protein ratio after a mild water stress in sunflower seedlings. Kraus *et al.* (1995) have also reported a decrease in chlorophyll content upon exposure to oxidative stress with comparatively higher chlorophyll content in tolerant wheat and maize genotypes under stress conditions than in susceptible ones. Sairam (1994) reported that under moisture stress, chlorophyll content and chlorophyll stability index was higher in tolerant wheat genotypes in comparison to the susceptible genotypes.

Gummuluru *et al*. (1989) reported that drought tolerant durum wheat had higher chlorophyll than the susceptible ones and maintained chlorophyll content to a higher level under stress. Pastori and Trippi (1993) showed that drought sensitive wheat cultivars (Leones) had greater loss of chlorophyll on moisture stress, when compared with the drought tolerant ones. Misra and Misra (1987) in their study of the effect of age and dehydration on greening of wheat leaves found that water stress inhibited chlorophyll synthesis and that the younger seedlings were more prone to stress than older ones. Baisak *et al*. (1994) also confirmed the decline in chlorophyll

content when the wheat leaves were subjected to water stress by incubation with polyethylene glycol solution. Gupta *et al.* (2000) reported that chlorophyll stability index can be a good screening parameter for drought tolerance/susceptibility.

Carotenoids, besides acting as a photo-receptive antenna pigment for photosynthesis, also exhibit a protective function against oxidative damage (Knox and Dodge 1985). Misra and Misra (1997) reported that carotenoids decreased markedly on induction of water stress. They further confirmed that under osmotic stress the activities of endogenous protective enzyme systems and the contents of ascorbic acid and carotenoid were negatively correlated with membrane lipid peroxidation via-a-vis plasma membrane permeability.

Zhang and Kirkham (1996) in a comparative study of $C_3$ and $C_4$ plants observed decrease in carotenoid content of sorghum on drought while there was no change in sunflower plants. Tolerant cultivars. of wheat were found to retain higher contents of both chlorophyll and carotenoids than the susceptible ones, during post anthesis water stress (Sairam *et al.* 98).

### *2.10 Role of polyamines in inducing drought tolerance*

Polyamines play a pivotal role in altering plant responses under drought, salinity and thermal oscillation. Many type of stresses produced characteristic metabolic changes and resulted in the accumulation of a wide array of osmoregulatory solute viz. sugar, proline, glycine betaine, polyamines etc. Stabilization of membranes and minimization of water stress of various kinds of cells are of the several known physiological effect of polyamines in plant system (Galston 1983, Sharma 1999, Gupta *et al*. 2003b)

The protective role of polyamines against stress can be explained by their polycationic nature, their ability to bind with photosynthetic membrane, resulting in confirmational changes and stabilizing of the membrane acting as free radical scavanger (Groppa *et al*. 2001). Spermidine induced drought tolerance in *Zea mays* by greatly promoting the activities of SOD (superoxide dismutase) and POX (peroxidase), photosynthetic intensity but reduced stomatal resistance (Sang *et al*.

1996). Xu *et al.* (1995) reported that water stress increased MDA content and permeability of plasma lemma and decreased linolenic acid content in expanding flag leaves of wheat but when flag leaves were sprayed with 500 ppm spermine, the lipid peroxidation was decreased.

Uprety and Murti (1999) reported that polyamines (putrescine, spermidine and spermine) prevented the water stressed induced decline in relative water in pea and helped in the maintenance of better water balance. Yanling *et al*. (1998) observed an increased endogenous content of putrescine by the application of spermine in wheat seedlings under water stress. Toderov *et al.* (1998) also reported that putrescine increased the dry weight of maize seedlings grown under different water regimes. In tomato, water stress caused the accumulation of putrescine, spermidine and spermine but after rewatering, the level of polyamines returned to that of control plants. No varietal differences in dynamics of polyamine accumulation under water stress were noted (Kubis and Krzywanski 1991). In barley, accumulation of polyamines and increased activity of proteases were found as a result of water stress. No clear relationship was found between the level of polyamines and the levels of protease activity (Kubis and Krzywanski 1991). In maize, spermidine treatment promoted superoxide dismutase and peroxidase activity and photosynthetic activity but reduced stomatal resistance (Sang *et al*. 1996). Sharma (2001) have reviewed the role of polyamines in agricultural horticultural crops. In rice, grain filling and 1000 grain weight were positively correlated with polyamines content, particularly with spermine and spermidine. Spermidine and spermine were higher in rice cultivars with good grain filling characteristics (Yang *et al*. 1996). In soybean foliar spray of $10^{-3}$ M polyamines (putrescine, spermidine and spermine) at 50 per cent flowering stage showed increasing trend on number of pods per plant, 100 seed weight, seed and oil yields. Spermine was found to be most effective (Sharma and Ali 1998). The putrescine also found to enhance the productivity under drought in wheat. Among the different mode of application seed treatment plus spray was found most effective (Gupta *et al.* 2003b).

*Chapter 3.*

# Material and Methods

The experiments were conducted in laboratory, cage house and NARP field at SKN College of Agriculture, Jobner during Rabi 2001-02 and 2002-03 to investigate "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)". In order to achieve the objectives of present investigation, the experiments were planned and executed as described below:

## 3.1 Laboratory Experiments

### Expt. 3.1.A. Effect of water stress on antioxidant enzyme activity and lipid peroxidation in contrasting wheat genotypes

Seeds of two wheat genotypes C-306 (drought tolerant) and HD 2329 ( drought sensitive and widely adapted) of uniform size were selected and surface sterilized with 0.1 per cent mercuric chloride solution for two minutes and then washed thoroughly with sterilized distilled water. The seeds were sown in individual cells of root trainers available in growth chamber. 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.per cents. Water stress conditions were created by irrigating these cells with PEG 6000 (10 and 20 %). Cells of the root trainers irrigated with distilled water were served as control. Following observations were recorded in 9, 12 and 15 days old seedlings.

Superoxide dismutase
Catalase
Peroxidase
MDA content
$H_2O_2$ content

Seedlings of the same age i.e. 9, 12 and 15 days old seedlings with all the above treatments were dried in oven and following nutrients were measured.

Nitrogen
Phosphorous
Potassium
Calcium
Manganese
Iron
Copper

**Expt. 3.1 B. Changes in antioxidant enzyme activity and lipid peroxidation in relation to putrescine induced drought tolerance in wheat**

The seeds of same genotypes of wheat i.e. C-306 and HD-2329 were soaked in putrescine dihydrogen chloride (0.1 mM) for six hours and then air-dried. The seed were sown in cells of root trainers available in growth chamber as described above. For control, the seeds were soaked in distilled water and sown in root trainers under similar soil and environmental conditions. The water stress conditions were created

by irrigating the cells of root trainers with water containing 20% PEG-6000. Following observations were recorded in 9, 12 and 15 days old seedlings.

Superoxide dismutase
Catalase
Peroxidase
MDA content
$H_2O_2$ content

Seedlings of the same age i.e. 9, 12 and 15 days old seedlings with all the above treatments were dried in oven and following nutrients were measured:

Nitrogen
Phosphorous
Potassium
Calcium
Manganese
Iron
Copper

**Expt. 3.1.C. Physiological attributes of drought tolerance in wheat**

Seeds of two wheat genotypes C-306 (drought tolerant) and HD 2329 (drought sensitive and widely adapted) of uniform size were selected and surface sterilized with 0.1 percent mercuric chloride solution for two minutes and then washed thoroughly with sterilized distilled water. The seeds were then transferred to sterilised petriplates lined with Whatman filter paper No. 1. Ten seeds of each genotype were placed in each petriplate and irrigated with 10.0 ml distilled water containing different concentrations of PEG 6000 i.e. 0, 10 and 20 percent. These petriplates were kept in a growth chamber fitted with fluorescent tubes. Temperature of the chamber was

maintained between 22±2°C. After seven days, the seedlings of both the genotypes were transferred to the individual cells of root trainer already available in the growth chamber under similar environmental conditions. The 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.per cents. Water stress conditions were created by irrigating these cells with PEG 6000 (10 and 20 percent). Cells of the root trainers irrigated with distilled water were served as control. Germination percentage was observed in petriplates at 24, 48 and 72 h after incubation. Following observations were recorded in 5, 7, 9, 12 and 15 days old seedlings.

Seedling growth
Seedling vigour index
Relative water content
Membrane stability index
Chlorophyll content
Sugars
Protein content

## 3.2 . Pot Experiment

*Physiological attributes of drought tolerance in wheat at different growth stages*

The same wheat (*Triticum aestivum* L.) genotypes i.e. C-306 (drought tolerant) and HD-2329 (drought sensitive and widely adapted) were sown in ceramic pots filled with sandy loamy soil and farm yard manure in 6:1 ratio. The soil has bulk density of 1.50 g $cm^{-3}$, pH 8.4, field capacity 11.8 and permanent wilting point 2.8 per cent. After thinning, five plants were maintained in each pot. Plants were watered as and when required to keep them fully turgid. Water stress conditions were created by withholding water supply for a uniform period of 8 days and thereafter the plants were

irrigated. Pots were saturated with water before starting the moisture stress treatment. The water stress treatments were given at tillering, anthesis and post anthesis stages. One hundred pots were used for each genotype. Soil samples were collected from control and stressed pots at different stages. The mean soil moisture content in control pots was 10.5 to 11.5 per cent whereas stressed pots varied from 7.93 to 8.98 in the year 2001-02. Similarly, in the year 2002-03, the soil moisture content varied between 11.5 to 13.0 per cent for control pots and 7.20 to 7.90 per cent in stressed pots in two genotypes. Following observations were recorded at tillering, anthesis and post anthesis stage between 10 to 12 AM on the top most fully expanded leaf:

Photosynthesis
Transpiration
Stomatal conductance
Internal $CO_2$ concentration
Water use efficiency
Relative water content
Membrane stability index
Chlorophyll content
Carotenoid content
Sugars
Proline

Plants of both the treatments were harvested at maturity and following observations were recorded:

Plant height
Leaf number
Leaf area
Number of effective tillers
Number of grains per spike
Grain weight per ear
Grain yield per plant

Biological yield per plant

Harvest index

3.3 Field Experiment

*Evaluation of the field efficacy of putrescine application in wheat*

In order to study the field applicability of putrescine, an experiment was conducted at NARP field, SKN College of Agriculture, Jobner in the rabi seasons of the year 2001-02 and 2002-03.

## Experimental conditions

Jobner is situated at an elevation of 420 meters above mean sea level at 26.6° N and 75.25° E. The climate of this zone is typically semiarid characterized by wide range of temperature both in winter (-2 to 10° C) and in summer (30 to 45° C). The normal rainfall of this tract varied between 400-500 mm, most of which receives from South East monsoon during July to September. Mean weekly weather parameters during two crop seasons collected from the college meteorological observatory have been presented in appendix-I. The soil used was sandy loam having bulk density 1.5 g $cm^{-3}$.

**Experimental details**

The experiment was laid out in randomized block design with three replications under non stress and water stress conditions. The layout of the experiment has been depicted in appendix-II and other details are given below.

| | |
|---|---|
| Genotype: | HD 2329 |
| Experimental conditions: | Non stress and water stress |

Treatments:

1. Control
2. Seed treatment (1.0 mM, 0.1 mM. 0.01 mM Putrescine).
3. One foliar spray (1.0 mM, 0.1 mM. 0.01 mM Putrescine).
4. Two foliar spray (1.0 mM, 0.1 mM. 0.01 mM Putrescine).

| | |
|---|---|
| Replications: | Three |
| Total number of treatments: | 10 x 2 = 20 |
| Total number of plots: | 30 x 2 = 60 |
| Plot size: | 3 m x 2 m |

## Field preparation

The field was initially plough by disc plough followed by cross harrowing and planking to bring the field into good tilth. A pre sowing irrigation was given for proper germination and establishment of the seedlings.

## Fertilizer application

A uniform basal dose of 90 Kg N $ha^{-1}$ through urea, 30 Kg $P_2O_5$ $ha^{-1}$ through single super phosphate and 30 kg $K_2O$ $ha^{-1}$ through muriate of potash were drilled prior to sowing. The remaining nitrogen was top dressed through urea and a light irrigation was applied to avoid volatilization losses and better absorption.

## Seed treatments and sowing

The seeds were soaked in different concentrations of putrescine dihydrochloride (1.0 mM, 0.1 mM. 0.01 mM Putrescine) for six hours (for seed soaking treatment)

followed by air-drying prior to sowing. For control, the seeds were soaked in distilled water. The treated seeds were sown at about 5 cm depth in rows of 25 cm apart.

## Spray treatments

The first spray of different concentrations of putrescine dihydrochloride was done at anthesis stage (6.2.02 and 8.2.03) and second spray was done at post anthesis stage (25.2.02 and 28.2.03). About 250 ml solution of each concentration were used in each plot and the control plants were sprayed with distilled water.

## Irrigations

Beside one pre sowing irrigation, six irrigations were given at different critical stages for non stressed plots while for stressed plots only three irrigation were given as:

| Irrigation | 2001-02 | | 2002-03 | |
|---|---|---|---|---|
| | Non stress | Stress | Non stress | Stress |
| I | 10.12.01 | 10.12.01 | 4.12.02 | 4.12.02 |
| II | 4.1.02 | - | 25.12.02 | - |
| III | 15.1.02 | - | 15.1.03 | - |
| IV | 23.1.02 | 23.1.02 | 5.2.03 | 5.2.03 |
| V | 11.2.02 | - | 26.2.03 | - |
| VI | 15.3.02 | 15.3.02 | 18.3.03 | 18.3.03 |

**Weeding and hoeing**

One weeding and hoeing was done manually (25.2.02 &, 3.1.03) to reduce weed competition.

## Harvesting and threshing

Each plot harvested separately in both the non-stress and stressed plots. Then harvested material of each plot was tied up in bundles and kept on threshing floor for sun drying. Threshing and winnowing was done manually separately for each plot and grain yield per plot was measured.

## Observations

Following observations were recorded after harvesting the crop:

Plant height
Leaf area per plant
Number of ears /m row
Number of grains/ ear
Grain yield
Biological yield
Harvest index
Test weight

## 3.4. Preparation of Test Solutions

## Putrescine solutions

Different concentrations of putrescine dihydrochloride (1.0 mM, 0.1 mM. 0.01 mM Putrescine) were prepared by dissolving required amount of putrescine (mol. wt. 161.1 g) in distilled water and neutralized with traces of 1M NaOH to pH 7.0. These concentrations were selected on the basis of previous studies (Gupta *et al.* 2003b) and preliminary trials. Foliar sprays were given at anthesis stage (one spray treatment) and at anthesis and post anthesis stages (two spray treatment). Twin 80 was used as adhesive during spray of putrescine.

## PEG 6000 solutions

10 and 20 per cent polyethylene glycol (PEG) 6000 solutions were prepared by 10 and 20 g polyethylene glycol (PEG 6000), respectively, in 100 ml distilled water.

# 3.5. Procedures and Techniques

### 3.5.1 Germination and Growth Parameters

#### 3.5.1.1 Germination percentage

Emergence of radical (about 2 mm length) was viewed as germination and germination percentage was calculated by counting the number of seeds germinated in each petriplate at 24, 48 and 72 hour after incubation.

#### 3.5.1.2. Seedling length

The shoot and root length were measured at 5, 7, 9, 12 and 15 days with the help of meter scale and thread.

### 3.5.1.3 Seedling vigour index

Seedling vigour index at 5, 7, 9, 12 and 15 days old seedlings were measured by the formula given by Singh and Kakralya (1995):

$$SVI\ (\%) = (Shoot\ Length + Root\ Length\ /100) \times Germination$$

## 3.5.2 Physiological Parameters

### 3.5.2.1 Relative water content

Fresh weight of the samples was taken and then kept in distilled water for 4 hours (Barrs and Weatherly 1992) to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surfaces of the samples. Dry weight was obtained after drying the samples in oven at 60°C till constant weight obtained. The relative water content (RWC) was then calculated by the formula given by Slavik (1974):

$$RWC\ (\%) = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} \times 100$$

### 3.5.2.2. Photosynthesis

The rate of photosynthesis ( μm $CO_2$ $m^{-2}s^{-1}$) was measured with the help of infra red gas analyzer (CID-301, USA). The net exchange of $CO_2$ between the leaf and atmosphere was measured directly by enclosing the top most expanded leaf in the assimilation chamber and the rate was monitored at which the $CO_2$ concentration changed over a definite time interval. The system automatically calculated the rate of photosynthesis on the basis of preloaded flow and leaf area as per the chamber used. Measurements were taken between 10.0-11.0 AM in triplicates for all the treatments.

### 3.5.2.3. Transpiration and stomatal conductance

The leaf transpiration rate (μg $H_2O$ $cm^{-2}s^{-1}$) and stomatal conductance (mmol $m^{-2}s^{-1}$) were measured directly with the help of infra red gas analyzer (CID 301, USA) on the same leaf as described for the photosynthesis.

## 3.5.2.4. Membrane stability index

The wheat membrane stability index (MSI) was determined according to the method of Premchand *et al*. (1990) and modified by Sairam (1994). Shoot portion (0.1 g) of different treatments and control were thoroughly washed in running tap water and double distilled water and thereafter placed in 10 ml of double distilled water at 40°C for 30 minutes. After the end of this period their electrical conductivity was recorded by conductivity bridge ($C_1$). Subsequently the same samples were placed on boiling water bath (100°C) for 10 min and their electrical conductivity was recorded as above ($C_2$). The membrane stability index (MSI) was calculated as:

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

### 3.5.2.5.Water use efficiency

Water use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration rates:

$$WUE = \frac{\text{Photosynthesis rate}}{\text{Transpiration rate}}$$

## 3.5.3 Metabolites

### 3.5.3.1 Photosynthetic pigments

Total chlorophyll content (the sum of chlorophyll ‘a’ and chlorophyll ‘b’) and carotenoids content were estimated according to the method of Arnon (1949). Sample extract was prepared from 100 mg of leaf with 10 ml of 85% acetone and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25 ml measuring cylinder. The residue was again re-extracted with 5 ml of acetone and centrifuged and then the supernatant was transferred to the measuring cylinder. The final volume of the supernatant was made to 20 ml with the acetone.

Finally, the optical density of chlorophyll ‘a’ and ‘b’ was measured at 663 and 645 nm. The optical density of same samples was measured at 450 nm for estimating the carotenoid contents. The total chlorophyll content in $mgl^{-1}$ was calculated by the formulae:

$$\text{Total chlorophyll (mg } l^{-1}) = 20.2\ A_{645} + 8.02\ A_{663}$$

Where,

$A_{645}$ and $A_{663}$ were absorption (optical density) of chlorophyll ‘b’ and ‘a’, respectively. From this value, chlorophyll content in mg $g^{-1}$ of fresh weight of the leaf sample was calculated using the expression:

$$\text{Total chlorophyll (mg } g^{-1}) = \frac{\text{Total chlorophyll (mg } l^{-1})}{1000} \times \frac{\text{Total volume of the extract}}{\text{Sample weight (g)}}$$

The Carotenoid content was calculated by using the following formula:

Carotenoid (mg $g^{-1}$)= D x V x F x 10/2500

Where, D is absorbance at 450 nm in a cuvette of 1 cm light path

V is volume of the orginal extract in ml

F is dilution factor

2500 is average extinction coefficient of the pigment.

**3.5.3.2. Total soluble sugars**

Total soluble sugars (mg $g^{-1}$ f. w.) were extracted in 5 ml of hot 80% ethanol from 100 mg of leaf sample using anthrone reagent (Dubois *et al.,* 1956). The homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was collected in a beaker and evaporated on a hot plate. Then the residue was dissolved in 10 ml $dH_2O$, which is called as the sample extract.

Sample extract of 0.2 ml was taken in a test tube and at the same time in a series of test tubes 0.2, 0.4, 0.6, 0.8 and 1.0 ml of glucose solutions were prepared by dissolving 10 mg of glucose in 100 ml of $dH_2O$. In each test tube, the volume was

made to 1.0 ml with $dH_2O$. A tube with 1.0 ml of $dH_2O$ was served as a control. In all the test tubes, 4 ml of anthrone reagent (2 mg/ml in conc. $H_2SO_4$) was added slowly and mixed carefully. Tubes were kept in boiling water bath for 10 minutes then brought to room temperature. Absorbance of blue green color was measured at 620 nm using spectrophotometer. The quantity of total soluble sugars in the 100 mg of leaf was then calculated using the standard curve.

**3.5.3.3. Total soluble proteins**

For extraction of soluble proteins (mg $g^{-1}$ f. w.), 100 mg of leaf sample was homogenised in phosphate buffer (0.1M, pH 7.5). The proteins were precipitated by adding 10 % trichloro acetic acid. The homogenate was centrifuged at 5000 rpm for 10 min and the precipitate was dissolved in 2N NaOH.. The supernatant was collected and final volume was made to 5 ml with the buffer. The proteins in the extract were estimated using the method of Lowry *et al.* (1951). An extract volume of 0.2 ml was taken in test tube and at the same time in a series of test tubes 0.2, 0.4, 0.6, 0.8 and 1.0 ml of protein solutions were prepared by dissolving 10 mg of bovin serum albumin (BSA) in 100 ml of saline solution. In each test tube the volume was made to 1.0 ml with d $H_2O$. A tube with 1.0 ml of $dH_2O$ served as a blank.

After adding 5 ml of alkaline solution in each test tube, the mixture was kept at room temperature for 10 min. This was followed by addition of 0.5 ml of diluted Folin-ciocalteau's reagent. The mixture was incubated at room temperature for 30 min under dark and the absorbance of the blue colour was measured at 620 nm using spectrophotometer. The quantity of proteins in the 100 mg of leaf was then calculated using the standard curve.

**Reagent preparation:**

**Reagent A:** 2% $Na_2CO_3$ in 0.1 M NaOH

**Reagent B:** 0.5% Copper sulphate ($CuSO_4.5H_2O$) in 1% Potassium Sodium tartrate.

**Reagent C:** Alkaline copper solution which was prepared by mixing reagent A and B in the ratio of 50:1 at the time of use.

**Reagent D:** The commercial Folin-ciocalteau reagent was diluted with equal volume of water.

#### 3.5.3.4. Proline

Free proline in leaves (mg $g^{-1}$ f. w.) was extracted and determined by the method of Bates *et al.* (1973). 100 mg Fresh leaf sample was homogenized in 5 ml of 3% aqueous sulphosalicylic acid and the supernatant was collected after centrifugation at 5000 rpm for 5 min. The volume of supernatant was made to 10 ml with the sulphosalicylic acid. The sample extract of 2 ml was taken in one test tube and at the same time in a series of test tubes 0.2, 0.4, 0.6, 0.8 and 1.0 ml of proline solutions were prepared by dissolving 10 mg of proline in 100 ml of 3 per cent sulphosalicylic acid. In each test tube, the volume was made to 2 ml with 3 % sulphosalicylic acid. A tube with 2 ml of sulphosalicylic acid was served as a control.

In all test tubes 2 ml ninhydrine reagent (1.25 g ninhydrine in 20 ml of 6 N orthophosphoric acid) and 2 ml glacial acetic acid were added and kept in boiling water bath at 100°C for 10 min and then cooled in ice bucket. Then, 4 ml toluene was added and mixed thoroughly by vortex mixer for 10 seconds. Optical density was measured at 520 nm against a reagent blank.using spectrophotometer. The quantity of free proline in the 100 mg of leaf was then calculated using the standard curve.

### 3.5.4 Antioxidants

#### 3.5.4.1. Superoxide dismutase activity

Enzyme extract for superoxide dismutase (SOD) and catalase was prepared by grinding 0.5 g leaf material with 10 ml of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The beri was filtered through cheesecloth and the

filtrate was centrifuged in a refrigerated centrifuge (IEC, India) for 15 min at 20,000 x g. The supernatant is referred to as enzyme extract. All operations were carried out at 4°C. Superoxide dismutase activity was estimated according to the method of Dhindhsa *et al.* (1981). The 3.0 ml reaction mixture contained 13 mM methionine, 25 mM nitroblue tetrazolium chloride (NBT), 0.l m M EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.1 ml enzyme extract. Reaction was started by adding 2 μM riboflavin and placing the tube below 2 x 15 W fluorescent lamp for 15 minutes. Reaction was stopped by switching off the light and covering the tube with block cloths. Tubes without enzyme develop maximum colour. A non irradiated complete reaction mixture did not develop colour and served as control. Absorbance was recorded at 560 nm and one unit of enzyme was taken as that quantity of enzyme, which reduced the absorbance reading 50% in comparison to the tubes lacking enzyme.

**Preparation of reagents**

1. 200 mM L-Methionine0.298 g of L-Methionine dissolved in $dH_2O$ and volume was made upto 10 ml.
2. 2.25 mM NBT: 0.0184 g of NBT (nitroblue tetrazolium) was dissolved in $dH_2O$ and volume was made up to 10 ml and kept air tight.
3. 60 μM Riboflavin: 0.0023 g of riboflavin was dissolved in $dH_2O$) and volume was made up to 100 ml and stored in amber coloured bottles.
4. 1.5 M $Na_2CO_3$: 1.59 g of $Na_2CO_3$ was dissolved in $dH_2O$ and volume was made up to 10 ml.
5. EDTA (3 mM): 87.675 mg dissolved in $dH_2O$ and volume was made up to 100 ml.

### 3.5.4.2. Catalase activity

Catalase (CAT) activity was assayed by measuring the disappearance of $H_2O_2$ according to Teranishi *et al.* (1974). The 3.0 ml reaction mixture contained 50 mM phosphate buffer (pH 7.0), 20 mM $H_2O_2$ and 0.1 ml diluted enzyme. The reaction was stopped after 5 minutes by the addition of 2 ml titanium reagent, which also formed colored complex with the residual $H_2O_2$. Aliquot was centrifuged at 10,000 g for 10 min and the absorbance of the supernatant was recorded at 410 nm in uv visible spectrophotometer.

**Preparation of reagents**

1. Potassium phosphate buffer: Potassium hydrogen phosphate (0.1 M) was prepared by dissolving 1.74 g in 100 ml $dH_2O$. Further, potassium dihydrogen phosphate (0.1 M) was prepared by dissolving 1.36 g in 100 ml $dH_2O$. Potassium hydrogen phosphate and potassium dihydrogen phosphate were mixed in the ratio of 84.16 and pH was adjusted to 7.5 with pH meter.
2. Titanium reagent: 1 g of titanium dioxide and 10 g of $K_2SO_4$ were mixed and digested with 150 ml concentrated $H_2SO_4$ for 2 h in a hot plate. The digested mixture was cooled and diluted to 1.5 litre with distilled water and used as titanium reagent.

### 3.5.4.3. Peroxidase activity

Enzyme extract for peroxidase (POX) activity was prepared by grinding 0.1 g of leaf material with 10 ml of prechilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA in a prechilled mortar and pestle. The beri was filtered through cheesecloth and the filtrate was centrifuged in a refrigerated centrifuge for 15 minutes at 20,000 g. All operations were carried out at 4°C. POX activity was estimated at 25°C in a cuvette containing 100 mM potassium phosphate buffer (pH 6.0), 0.01 M O-dianisidine 20 mM $H_2O_2$ and 0.1 ml diluted enzyme extract (10 times). The increase in absorbance was recorded at 470 nm over a period of 10

minute. One enzyme unit is an increase in absorbance unit per minutes per gram fresh weight of the samples (Castillo *et al*. 1984).

## Preparation of reagents

1. Potassium phosphate buffer (0.1 M): 3.40 gm potassium dihydrogen phosphate ($KH_2PO_4$) was dissolved in distilled water and pH was adjusted with KOH and total volume was made to 250 ml with distilled water.
2. O- dianisidine (0.01 M): 0.2448 g dissolved in 100 ml methanol (prepared fresh).
3. $H_2O_2$ (20 mM): 5 ml of 30% $H_2O_2$ was diluted to 200 ml with $dH_2O$.

### 3.5.4.4. MDA Content

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, following the method of Heath and Packer (1968). A leaf sample (0.5 g) was homogenized in 10 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 g for 5 minutes. To 1.0 ml aliquot of the supernatant 4.0 ml 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. After centrifugation at 10000 g for 10 min, 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 by its extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ and expressed as nmol MDA per gram fresh weight.

### 3.5.4.5. $H_2O_2$ Content

Sample preparation and $H_2O_2$ estimation was done as described by Mukherjee and Chaudhary (1983). Plant material (0.5 g) was homogenized in 10 ml of cold acetone. The homogenate was filtered through Whatman No. 1 filter paper. To whole of the extract 4.0 ml of titanium reagent was added followed by 5.0 ml of

concentrated ammonium solution to precipitate the peroxide titanium complex. After centrifugation for 5 min at 10000 xg the supernatant was discarded and residue was dissolved in 10 ml of 2N $H_2SO_4$. It was recentrifuged to remove the undissolved material and absorbance was recorded at 415 nm against water blank. Concentration of $H_2O_2$ was determined with the help of the standard curve plotted with known concentration of $H_2O_2$.

### 3.5.5. Nutrient Estimation

#### 3.5.5.1. Nitrogen

The nitrogen was estimated by digesting plant samples with sulphuric acid using hydrogen peroxide for removing black colour. Estimation of N was done by colorimetric method using Nesseler's reagent to develop colour (Snell and Snell 1949).

#### 3.5.5.2. Phosphorus

The phosphorus was estimated by digesting the plant samples with triacid mixture using ammonium vanadate solution for developing colour intensity by vando-molybdo phosphoric acid method (Jackson 1973).

#### 3.5.5.3. Potassium

The potassium was estimated by flame photometer method (Bhargava and Raghupati 1993).

#### 3.5.5.4. Calcium

The calcium content was determined by digesting the sample in diacid and fed to the atomic absorption spectrophotometer using hollow cathodes lamp of calcium (Bhargava and Raghupati 1993).

#### 3.5.5.5. Copper, manganese and iron

One gram oven dried plant samples were digested in 10 ml of triacid mixture and the digestion of sample was done till the content of flask become colourless. Then the sample extract is fed to atomic absorption spectrophotometer using the Cu, Mn, and Fe hallow cathodes lamps one by one and concentration were determined (Lindsay and Norwell 1978).

### 3.5.6. Growth and Yield Parameters

#### 3.5.6.1. Plant height

Plant height (cm) was measured from base to top of the plant directly by meter scale. In pot experiment height of five plants were measured randomly whereas in field experiment ten plants were taken. The average plant height was calculated finally.

#### 3.5.6.2. Number of leaves

In pot experiment five pots were selected randomly and their number of leaves was counted in each pot and then the average was calculated on per plant basis.

#### 3.5.6.3. Leaf area

Leaf area ($cm^2$) was measured with the help of leaf area meter (LiCOR 3000 USA). In pot experiment five plants were observed randomly whereas in field experiment ten plants were taken. The average leaf area was calculated finally.

#### 3.5.6.4. Number of ears

In pot experiment, the number of ears were counted from five plants selected randomly from each treatment and then the average was calculated. In field experiment, the total number of ears were counted from the plants grown in m row length.

#### 3.5.6.5. Grain yield

In pot experiment, plants were harvested and dried in oven till constant weight obtained. Then the average yield (g) was calculated per plant basis. In field experiment, plants were harvested at maturity. After threshing and winnowing, the grain yield obtained from each experimental unit was weighed and expressed in terms of q $ha^{-1}$.

**3.5.6.6 Biological yield**

The produce (grain plus straw) of the each experimental unit after harvest was sundried for five days, thereafter weighed and expressed in terms of q $ha^{-1}$.

**3.5.6.7. Harvest index**

The ratio of economic yield to biological yield was worked out to estimate harvest index (HI) by the formula of Singh and Stoskoff (1971):

Harvest Index (%) = (Economic Yield/Biological Yield) x 100

**3.5.6.8. Test weight**

1000 grains were counted from random samples and their weight was recorded.

3.5.7. Statistical Analysis

All the observations of laboratory and pot experiments were taken in triplicates and data were statistically analysed using complete randomized design (CRD). The data obtained from field experiment was analysed using randomized block design (RBD). Standard error of mean (SEm±) and critical difference (CD) were calculated (Raghavrao 1983).

$$SEm\pm = \sqrt{\frac{2\,MSE}{r}}$$

$$CD = SEm \times t_{\infty}$$

Where, MSE is mean square of error, r is replication and $t_{\infty}$ is table value of 't' at 5 or 1 % level of significance with error degree of freedom.

# *Chapter 4.*

# Experimental Results

Research findings arising out from the study entitled "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)" conducted during rabi seasons 2001-02 and 2002-03 under laboartory, pot and field conditions at S.K.N. college of Agriculture, Jobner are presented and described in this chapter.

Experiment No.1.

## EFFECT OF WATER STRESS ON ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN WHEAT GENOTYPES

**Superoxide dismutase**

Effect of water stress on superoxide dismutase (SOD) activity in two wheat genotypes recorded in 9, 12 and 15 days old seedlings have been presented in table

4.1. Results showed that the genotypic variation in SOD activity was significant only at 12 day. SOD activity increased significantly under PEG induced water stress in 9, 12 and 15 days old seedlings in both the genotypes.

Further, the SOD activity increased linearly with increasing concentrations of polyethylene glycol (PEG) in all the observations. Significant differences were also observed on treatment x genotype interaction. Under non stress conditions the SOD activity was higher in HD 2329 but under water stress condition higher magnitude was reported in C-306.

Effect of PEG treatment, irrespective of the genotypes, revealed a linear enhancement in SOD activity in 9, 12 and 15 days old seedlings (Table 4.1)

**Peroxidase**

A reference to data recorded on peroxidase activity showed that there was significant difference in its activity between two genotypes at 9, 12 and 15 days old seedling (Table 4.1). The genotype C-306 showed higher peroxidase activity than HD 2329. The peroxidase activity also increased with the advancement of days in both the genotypes.

The peroxidase activity increased by PEG induced water stress at 9, 12 and 15th days. The magnitude of increases was reported to increase with increasing PEG concentration (Table 4.1).

Significant differences were also observed on genotype x treatment interaction. Under both non stress and water stress conditions higher peroxidase activity was observed in C-306 all the time of observations. The percent increase in peroxidase activity on account of water stress was higher in C-306 as compared to HD 2329 (Table 4.1).

**Catalase**

Data presented in table 4.1 showed that catalase activity differed significantly with respect to genotype, treatment and their interaction in 9 and $15^{th}$ day old seedlings. Genotype C-306 exhibited higher catalase activity than HD 2329 both under non stress and water stress conditions.

Significant increase in catalase activity on account of 10% PEG induced water stress was observed which further increased with 20% PEG treatment. The trend of increment in it activity was similar in two genotypes; however, the magnitude was variable (Table 4.1).

**MDA content**

Data depicted in table 4.2 show the effect of water stress on malondialdehyde (MDA) content in two wheat genotypes in 9, 12 and 15 days old seedlings. There was a significant difference in MDA content between two genotypes. HD 2329 exhibited higher MDA content both under control and PEG induced water stress conditions.

The water stress significantly increased the MDA content. An increasing magnitude of MDA content was reported with increasing PEG concentrations. However, the percent enhancement in its content was lower from 10% PEG to 20% PEG as compared with the control to 10% PEG (Table 4.2).

Significant difference in MDA content with respect to genotype x treatment interaction was observed in 9, 12 and 15 days old seedlings. It was also noted that MDA content was higher in susceptible genotype HD 2329 under control conditions which further increased at faster rate over C-306 under water stress conditions (Table 4.2).

**$H_2O_2$ Content**

The results obtained on hydrogen peroxide ($H_2O_2$) content showed that its content differed significantly with respect to treatment x genotype interaction in 9, 12 and 15 days old seedlings. HD 2329 exhibited higher $H_2O_2$ content than C-306 under non stress and water stress conditions. The percent increase in $H_2O_2$ content on account of water stress was also higher in HD 2329 than C-306 (Table 4.2).

Water stress significantly increased $H_2O_2$ content. The maximum increase in $H_2O_2$content was reported with PEG 20%. Genotypic variations, irrespective of the treatments, revealed that tolerant genotype C-306 maintained lower $H_2O_2$ content at all the time of observations (Table 4.2).

**Nitrogen content**

Table 4.3 shows the nitrogen content of two wheat genotypes in 9, 12 and 15 days old seedlings. It indicates that the nitrogen content differed significantly with respect to treatment, genotypes and their interaction. It was further observed that nitrogen content varied non significantly under control conditions but under PEG induced water stress, C-306 exhibited significantly higher nitrogen content over HD 2329. The trend was almost similar at all the three stages of observations.

Water stress significantly decreased the nitrogen content in both the genotypes. The decrease was found linear with increasing PEG concentrations. The genotypic differences were also observed significant with respect to nitrogen content. HD 2329 exhibited higher nitrogen content under control condition but under water stress C-306 retained more nitrogen content (Table 4.3).

**Phosphorus content**

As indicated in table 4.3, non significant difference in phosphorus content was observed with respect to genotype x treatment interaction. Phosphorus content was observed higher in C-306 over HD 2329 in most of the observations. PEG induced water stress conditions significantly decreased phosphorus content in 9, 12 and 15 days old seedlings. The maximum decrease was reported with PEG 20%.

Genotypic difference in phosphorus content was observed non significant in 9 12 and 15 days old wheat seedlings. Genotype C 306 exhibited higher phosphorus content in comparison to HD 2329 in most of the observations (Table 4.3).

**Potassium content**

Effect of water stress on potassium content in two wheat genotypes taken in 9, 12 and 15 days old seedlings has been presented in table 4.4. Genotype x treatment interaction shows significant differences only in 12 and 15 days old seedlings. It was higher in C-306 under control as well as water stress conditions. In 9 days old seedlings also, C-306 showed higher potassium content over HD 2329 but statistically it was non significant.

Potassium content increased on account of water stress at 9, 12 and 15 days old seedlings. Maximum increase in potassium content was observed with 20% PEG at all the stages. Genotypic difference in potassium content, irrespective of treatment,

was also found significant. Genotype C-306 exhibited higher potassium content than HD 2329 in 9, 12 as well as 15 days old wheat seedlings (Table 4.4).

**Calcium content**

A reference to data on calcium content with respect to genotype x treatment interaction revealed that HD 2329 exhibited higher calcium content in 9, 12 and 15 days old wheat seedlings. However, it was statistically significant only in 15 days old seedlings.

Genotypic difference in calcium content was found non significant at all the three stages with lower values in C-306.

A linear decrease in calcium content was observed on account of increasing PEG induced water stress. Maximum decrease in calcium content was recorded with PEG 20% in both genotypes (Table 4.4).

**Iron content**

Table 4.4 shows the effect of water stress on micronutrient content in two wheat genotypes at 9, 12 and 15 days old seedlings. The iron content differed significantly with respect to treatment and genotypes but their interaction showed non significant differences only in 12 and 15 days old seedlings.

The two genotypes differed significantly with respect to iron content. In general, genotype C-306 exhibited higher iron content than HD 2329. PEG induced water stress significantly decreased the iron content in both the genotypes. The C-306 retained higher iron content under both the conditions. The percent decrease in iron content with PEG induced water was more in HD 2329 and maximum decrease was reported with 20% PEG (Table 4.5).

**Manganese content**

Data on manganese content, depicted in table 4.5, show significant difference with respect to treatment x genotype interaction in 12 and 15 days old wheat seedlings. Genotypic variations were also found significant. In general, C-306 had higher manganese content than HD 2329.

Manganese content was found to decrease on account of water stress. The decline in manganese content on account of waters stress, irrespective of genotype, was found significant at 20% PEG in 12 and 15 days old seedlings whereas in 9 days old seedlings it was non significant

**Copper content**

The data presented in table 4.5 show that the copper content differed non significantly with respect to genotype and genotype x treatment interaction. The two genotypes also differed non significantly with respect to copper content. In general, C-306 exhibited higher copper content than HD 2329.

Water stress caused increase in copper content significantly in 9, 12 and 15 days old seedlings. Maximum increase in copper content was recorded with PEG 20%. The trend was almost similar in both the genotypes. However the magnitude was variable (Table 4.5).

## *Experiment No. 2*

**Antioxidative Enzyme System and Lipid Peroxidation in Relation to Putrescine Induced Drought Tolerance in Wheat**

**Superoxide dismutase**

Table 4.6 shows the superoxide dismutase, peroxidase and catalase activities of 9, 12 and 15 days old seedlings in relation to putrescine induced drought tolerance in wheat. Differences in superoxide dismutase (SOD) activity were significant in response to treatment, genotype and their interactions. It was further observed that SOD activity was found to increase by putrescine in both the genotypes under control condition, albeit, the differences were generally non significant. The SOD activity increased significantly by PEG induced water stress in both the genotypes at 9, 12 and 15 days (Table 4.6).

Genotype C-306 exhibited significantly higher SOD activity under water stress conditions in comparison to HD 2329. Putrescine under water stress conditions further enhanced the SOD activity in both the genotypes. However, the percent increase was more in C-306 than HD 2329 at all the stages (Table 4.6).

**Peroxidase**

Data presented in table 4.6 show that the peroxidase activity increased with advancement of days. Its activity increased significantly under water stress conditions in both the genotypes. The genotype C-306 exhibited higher peroxidase activity than HD 2329, particularly under water stress conditions. Putrescine alleviated the effect of water stress by increasing its activity both under non stress and water stress conditions in both the genotypes but under non stress conditions the increase was found non significant.

Genotypic variations, irrespective of treatments, were found significant with higher values of peroxidase activity in C-306 over HD 2329 at all the stages. Further, putrescine alone as well as in combination with PEG 20% enhanced the peroxidase activity. However, it was not significant at all the time of observations (Table 4.6).

**Catalase**

The differences in catalase activity were significant with respect to genotype and treatment but their interaction was found significant only in 12 and 15 days old seedlings (Table 4.6). Genotype C-306 exhibited higher catalase activity under control and water stress conditions. The enhanced catalase activity was reported by putrescine both under non stress and water stress conditions. However, the percent increase was more in water stress condition than non stress condition (Table 4.6).

Putrescine application, irrespective of genotypes, increased the catalase activity with a very high magnitude under water stress conditions than under control conditions. Data on genotypic variation, irrespective of the treatments, showed that C-306 exhibited significantly higher catalase activity over HD 2329 in 9, 12 as well as 15 days old seedlings (Table 4.6).

**MDA Content**

It is evident from the data presented in table 4.7 that the malondialdehyde content produced rapidly during lipid peroxidation on account of PEG induced water stress in 9, 12 and 15 days old wheat seedlings. The susceptible genotype HD 2329 exhibited higher MDA content both under non stress and water stress conditions.

The MDA content has been alleviated significantly by putrescine under water stress conditions. A non significant reduction in MDA content was observed by putrescine under non stress conditions in both the genotypes. It was further noted that the MDA content increased under water stress conditions in comparison to control but putrescine application decreased its content to some extent particularly with 20% PEG induced water stress.

Genotypic response, irrespective of treatment, revealed a significantly higher MDA content in HD 2329 over C-306 in 9, 12 and 15 days old seedlings (Table 4.7).

**$H_2O_2$ Content**

Results presented in table 4.7 show significant difference in hydrogen peroxide ($H_2O_2$) content in respect to genotype, treatment and their interaction. The genotype HD 2329 exhibited higher $H_2O_2$ content both under non stress and water stress conditions. The $H_2O_2$ content increased significantly on account of PEG induced water stress.

Putrescine reduced the effect of water stress by reducing $H_2O_2$ content under non stress and water stress conditions. It was also noted that putrescine retained the $H_2O_2$ content to some extent only in plants treated with 20% PEG. Genotypic variability again found significant with higher $H_2O_2$ content in HD 2329 over C-306 at all the stages (Table 4.7).

**Nitrogen content**

Table 4.8 shows the changes in nitrogen content in relation to putrescine induced drought tolerant in wheat. It indicate that the two wheat genotypes differed significantly with respect to nitrogen content at 9 and 12 days old seedlings whereas in 15 days old seedlings, the difference was significant.

The nitrogen content decreased significantly on account of PEG induced water stress in both the genotypes. The genotype C-306 showed significantly higher nitrogen content than HD 2329 under water stress conditions. The genotypic variations, irrespective of treatments, were found significant only in 15 days old seedlings.

Putrescine increased the nitrogen content significantly under non stress conditions in both genotypes but under water stress condition putrescine decreased the nitrogen content when compared with control but significantly higher nitrogen content was reported when compared with 20% PEG (Table 4.8).

**Phosphorus content**

A reference to data presented in table 4.8 show that the phosphorus content differed significantly with respect to treatment but genotype and genotype x treatment interactions show non significant differences. Phosphorus content decreased significantly on account of PEG induced water stress.

Genotype C-306 has higher phosphorus content than HD 2329. However, the difference was statistically non significant. Irrespective of genotypes, putrescine increased the phosphorus content under non stress condition but under water stress condition it tried to alleviate the effect of water stress by increasing phosphorus content to some extent when compared with 20% PEG.

**Potassium content**

The genotype, treatment and their interaction differed significantly with respect to potassium content. Potassium content increased significantly on account of water stress in 9, 12 and 15 days old seedlings in both the genotypes. Genotype C-306 exhibited significantly higher potassium content than HD 2329 both under non stress and water stress conditions (Table 4.9).

Putrescine increased the potassium content both under non stress and water stress conditions in both the genotypes. However, the magnitude of increase was reported higher under water than non stress conditions (Table 4.9).

**Calcium content**

Genotype x treatment interaction data depicted in table 4.9 show non significant differences with respect to calcium content in 9 and 15 days old seedlings. The calcium content decreased on account of water stress. Putrescine increased the calcium content particularly under water stress conditions. Genotypic variations in

calcium content, irrespective of treatments, were found significant only in 12 and 15 days old wheat seedlings (Table 4.9).

**Iron content**

Iron content differed significantly with respect to treatment and genotype but their interaction shows significant difference only in 9 days old seedlings. The iron content decreased significantly on account of water stress in both the genotypes. Genotype C-306 exhibited higher iron content both under non stress and water stress conditions. Effect of genotypes, irrespective of treatments, also revealed a higher iron content of C-306 over HD 2329 (Table 4.10).

Putrescine increased the iron content significantly under non stress conditions. Although under water stress condition a significant decrease in iron content was reported by putrescine but when compared with PEG induced water stress a significant increase in iron content was recorded (Table 4.10).

**Manganese content**

The data presented in table 4.10 indicate significant differences in manganese content with respect to genotype and treatment but their interaction showed non significant differences.

The two genotypes differed significantly with respect to manganese content. The genotype C-306 has higher manganese content than HD 2329 both under non stress and water stress conditions. Manganese content decreased significantly on account of PEG induced water stress. Putrescine again increased the manganese content significantly both under non stress and PEG induced water stress conditions. However, the statistical significance was variable (Table 4.10).

**Copper content**

Data presented in table 4.10 show that the copper content differed significantly with regards to genotype and treatment but their interactions were found non significant in most observations. The copper content increased significantly with PEG induced water stress in 9, 12 and 15 days old seedlings. Putrescine further

enhanced the copper content significantly both under non stress and water stress condition, however, magnitude was higher under water stress conditions.

The genotypic variability, irrespective of treatments, was found significant in 12 and 15 days old wheat seedlings. Its contents were recorded higher in HD 2329 over C-306 (Table 4.10).

## EXPERIMENT NO. 3.

### Physiological Attributes of Drought Tolerance in Wheat Seedlings

#### Germination percentage

Table 4.11 shows the effect of polyethylene glycol (PEG) induced water stress on germination percentage in two wheat genotypes. The results indicate that germination percentage increased with advancement in the time of sowing. A non significant difference was observed in germination percentage with respect to genotype and genotype x treatment interaction.

The two genotypes differed non significantly. However, the genotype C-306 reflected higher germination percentage at 24, 48 and 72 hours than HD 2329 under both non stress and water stress conditions (Table 4.11).

The germination percentage decreased significantly by PEG induced water stress. Maximum decrease in germination percentage was reported with PEG 20% in both the genotypes (Table 4.11).

#### Root length

Data collected on root length of two wheat genotypes in 5, 7, 9, 12 and 15 days old seedlings have been depicted in table 4.12. Results analysed as genotype x water stress interaction revealed that water stress conditions reduced the root length linealy with increasing PEG concentration. The significant reduction in root length was pronounced after 9 days of germination. It was also noted that the percent

reduction in root length on account of water stress was higher in HD 2329 as compared to C-306.

Effect of PEG treatments, irrespective of genotypes, revealed a drastic reduction in root length at 10% PEG which further reduced significantly under 20% PEG conditions. The genotypic variation, irrespective of the treatments, was found significant with higher root length of C-306 at all the five stages of observations (Table 4.12).

**Shoot length**

A reference to data presented in table 4.13 show the effect of PEG induced water stress on shoot length in 5, 7, 9, 12 and 15 days old wheat seedlings. Significant differences in shoot length with respect to genotype and genotype x treatment interaction have been observed in 9, 12 and 15 days old seedlings.

Shoot length increased with advancement of stage in both the genotypes. PEG induced water stress reduced shoot length significantly at all the days of observations. The maximum decrease in seedling length was recorded with PEG 20% in both the genotypes (Table 4.13).

A significant genotypic difference was observed at 9, 12 and 15 days. In general, C-306 exhibited higher shoot length under control as well as water stress conditions (Table 4.13).

**Seedling vigour index**

Table 4.14 shows the seedling vigour index of two wheat genotypes in 5, 7, 9, 12 and 15 days old seedlings. Genotype x treatment interaction revealed that PEG induced water stress reduced the seedling vigour index significantly in both the genotypes but C-306 maintained higher index in all the observations.

Effect of PEG, irrespective of genotypes, indicate a significant reduction in seedling vigour index at 10% PEG which further reduced with 20% PEG. The magnitude of reduction was higher from control to 10% PEG as compared to 10% to 20% PEG (Table 4.14).

The genotypic variations, irrespective of treatment were also found significant. C-306 always exhibited significantlt higher seedling vigour index over HD 2329 (Table 4.14).

**Relative water content**

Effect of PEG induced water stress on relative water content in two wheat genotypes has been shown in table 4.15. The results indicate that the relative water content differed significantly with respect to treatment, genotype and their interaction.

Water stress conditions significantly reduced the relative water content in both the genotypes. The reduction was linear with increasing concentrations of PEG. Maximum decrease in this parameter was recorded with 20% PEG. Under control condition, genotype HD 2329 exhibited higher relative water content than C-306 but under PEG induced water stress a reverse trend was noticed (Table 4.15).

Genotypic variations, irrespective of treatments, were found significant except in 5 days old seedlings. The values of relative water content were higher in C-306 at all the time of observations (Table 4.15).

***Membrane stability index***

The data depicted in table 4.16 show the effect of PEG induced water stress on membrane stability index in two wheat genotypes in 5, 7, 9, 12 and 15 day old seedling.

It has been noted that the two wheat genotypes differed significantly in membrane stability index at all the stages. In general, the genotype C-306 exhibited higher membrane stability index both under control and water stress conditions.

Water stress significantly decreased the membrane stability index in both genotypes but percent decrease in membrane stability index was more in HD 2329. The maximum decrease in this parameter was reported with PEG 20% in both the genotypes (Table 4.16).

**Sugar content**

As mentioned in table 4.17, the total soluble sugar content found to differ significantly with respect to water stress treatments. The sugar content increased linearly with increasing PEG concentrations. The maximum increase in sugar content was reported with 20% PEG in both the genotypes.

Genotype and genotype x treatment interaction also differed significantly. The genotype C-306 exhibited higher sugar content in comparison to HD 2329 both under non stress and water stress conditions. Cumulative effect of genotypes, irrespective of treatments, also reflected significantly higher sugar content of C-306 at all the time of observations (Table 4.17).

**Protein content**

A reference to data presented in table 4.18 shows that the protein content differed significantly with respect to treatment, genotype and their interaction. Protein content increased with advancement of days.

Water stress significantly decreased the protein content in both the genotypes. Decrease in protein content was recorded with increasing PEG concentrations. Maximum decrease in protein content was reported with 20% PEG.

The two genotypes exhibited significant differences with respect to protein content. Under non stress conditions genotype HD 2329 showed higher protein content but under water stress it was higher in C-306. Overall, the contents of proteins were higher in C-306 in all the observations (Table 4.18).

**Chlorophyll content**

Data depicted in table 4.19 reveal the chlorophyll content in 5, 7, 9, 12 and 15 days old wheat seedlings. Interaction of genotype and treatment showed that the PEG induced water stress conditions reduced the chlorophyll content at all the stages. However, the reduction was significant only in 15 days old seedlings. Perusal of data further revealed that the percent reduction in this parameter was higher in HD 2329 than C-306 in all the observations.

Genotypic variations, irrespective of water stress, showed that C-306 always exhibited higher chlorophyll content. However, it was statistically significant only at later stages i.e. 12 and 15 days old seedlings. The PEG induced water stress, irrespective of the genotypes, reduced chlorophyll content significantly with 10 as well as 20% PEG. The reduction was linear with increasing the concentration of PEG (Table 4.19).

**EXPERIMENT NO. 4.**

## Physiological Attributes of Drought Tolerance in Wheat Genotypes at Different Growth Stages

### Photosynthesis

Changes in the rate of photosynthesis of flag leaves of two wheat genotypes for the crop season 2001-2002 and 2002-2003 and their pooled analysis have been presented in the table 4.20. It can be noted from the table that genotypes, irrespective of the treatment, differed non-significantly in photosynthesis at tillering, anthesis and post anthesis stages in the year 2001-2002 as well as 2002-03.

The water stress significantly reduced the rate of photosynthesis at all the three stages during both the experimental years. Interaction between genotype x treatment showed that there was a significant reduction in photosynthesis rate of both the genotypes on account of water stress conditions. The percent reduction in photosynthesis due to water stress was lesser in case of C-306 than HD 2329 (Table 4.20).

It was also noted that the rate of photosynthesis was maximum at anthesis stage which was followed by tillering stage. It was minimum at post anthesis stage. The trend was almost similar in both the years of experiments and the same has been exhibited in pooled analysis (Table 4.20).

**Transpiration**

Data presented in table 4.21 show the effect of water stress on transpiration of flag leaf in two wheat genotypes over two seasons i.e. 2001-02 and 2002-03 as well as their pooled mean. The rate of transpiration decreased with the advancement of maturity. The transpiration rate of two genotypes, irrespective of treatments, was found to differ non significantly at three developmental stages of both the years.

The transpiration rate differed significantly with respect to stress and stress x genotype interaction. HD 2329 showed higher rate of transpiration under non stress condition but under water stress condition the rate of transpiration was higher in C-306 during both the years. Further, the percent decrease in rate of transpiration was more in HD 2329 than C-306.

Water stress, irrespective of the genotypes, significantly decreased the rate of transpiration at all the stages. The trend was almost similar in both the years of experimentation as well as in pooled analysis (Table 4.21).

**Stomatal conductance**

Stomatal conductance of two wheat genotypes observed during 2001-02 and 2002-03 and their pooled analysis have been depicted in the table 4.22. The stomatal conductance decreased with the advancement of maturity. Maximum stomatal conducatcnce was recorded at tillering stage over the two years of experiments.

Water stress significantly decreased the stomatal conductance in both the genotypes at all the three stages. The pattern of reduction in stomatal conductance was similar in two genotypes but the percent reduction in stomatal conductance on account of water stress was more in HD 2329 than C-306 in both the years.

Stomatal conductance did not differ significantly with respect to genotype in all the observations except flowering stage of the year 2001-02. However, HD 2329 showed higher rate of stomatal conductance than C-306 (Table 4.22).

**Internal $CO_2$ concentration**

The internal $CO_2$ concentration measured at the time of photosynthesis has been shown in table 4.23. Data on genotype x treatment interaction showed that

internal $CO_2$ concentraion was higher under water stress conditions in both the genotypes at tillering, anthesis as well as post anthesis stages of two years experimentation i.e. 2001-02 and 2002-3 as well as pooled means. It was further observed that internal $CO_2$ concentration of C-306 was higher under control conditions whereas under water stress conditions, it was higher in HD 2329. The pooled analysis also shows the similar trend.

The genotypic response, irrespective of treatment, exhibited that HD 2329 maintained a significantly higher concentration of internal $CO_2$ at all the three stages of both the years. Similarly, the cumulative effect of water stress, irrespective of genotypes, showed that the internal $CO_2$ concentration was significantly higher under water stress conditions as compared with control conditions (Table 4.23).

### *Membrane stability index*

Table 4.24 shows the effect of water stress on membrane stability index of two wheat genotypes in two experimental years i.e. 2001-02 and 2002-03. The pooled analysis of membrane stability index has also been presented in this table.

Membrane stability index differed significantly with respect to treatment, genotype and treatment x genotype interaction in both the experimental years. Water stress significantly decreased the membrane stability index in both the genotypes. However, the percent decrease in membrane stability index was more in HD 2329 in comparison to C-306 in both the years (Table 4.24).

The genotypic variations in membrane stability index, irrespective of treatment were found significant. The genotype C-306 exhibited higher membrane stability index in comparison to HD 2329 in year 2001-02 as well as 2002-03 (Table 4.24).

It has also been noted that the membrane stability index decreased with advancement of maturity in both genotypes. However, its percent reduction with age was higher under water stress conditions than control (Table 4.24).

**Relative water content**

A reference to data presented in table 4.25 shows that the relative water content differed significantly with respect to stress, genotype and stress x genotype interaction in both the experimental years.

A significant decrease in relative water content was observed on account of water stress at all the three stages. In general, C-306 maintained significantly higher relative water content over HD 2329 under control as well as water stress conditions.

Relative water content decreased with the advancement of age. Maximum relative water content was reported at tillering stage. Genotypic variation, irrespective of treatments, showed that C-306 exhibited higher relative water content than HD 2329 in all the observations (Table 4.25).

***Water use efficiency***

Data on effect of water stress on water use efficiency have been depicted in table 4.26. Results showed that it was higher in C-306 under non stress and water stress conditions during the years 2001-02 as well as 2002-03. The same has also been reflected in pooled means. Genotype x interaction data revealed that the reduction in water use efficiency on account of water stress was significant in all the cases except in HD 2329 at tillering stage of the year 2001-02.

Genotypic variations, irrespective of treatments, were found significant. C-306 maintained higher water use efficiency at all the stages in 2001-02 and 2002-03.

Decrease in water use efficiency under water stress conditions, irrespective of genotypes, was found non significant at tillering stage of the year 2001-02. In other observations, it was found significant (Table 4.26).

**Chlorophyll content**

Table 4.27 shows the chlorophyll content of two wheat genotypes under non stress and water stress conditions in the year 2001-02 and 2002-03.

Data show that chlorophyll content differed significantly with respect to treatment, treatment x genotype interaction but non significant differences with respect to genotype at anthesis and post anthesis stages during both the experimental years.

Maximum chlorophyll content was reported at anthesis stage under non stress condition while under water stress condition maximum chlorophyll content was reported at tillering stage during both the years. HD 2329 showed higher chlorophyll content under non stress conditions while under water stress conditions it was higher in C-306 .

Genotypic differences, irrespective of treatment, was found non significant at anthesis and post anthesis stages during both the years. Water stress, irrespective of genotypes, also decreased the chlorophyll content in all the observations (Table 4.27).

**Carotenoid content**

Data on carotenoid content of two wheat genotypes at different stage have been present in table 4.28. The carotenoid content decreased with the advancement of age in both the genotypes under both the conditions.

Water stress significantly reduced the carotenoid content in both the genotypes at both the experimental years. The percent reduction in carotenoid content was observed higher in HD 2329 as compared with C 306 in all the observations.

The genotypic variations, irrespective of treatments, were found significant only at tillering stage in both the years (Table 4.28).

**Soluble sugar content**

Data on soluble sugar content of flag leaf measured at tillering, anthesis and post anthesis stages have been presented in table 4.29. The sugar content was found maximum at post anthesis stages of both the years.

Sugar content was observed higher in HD 2329 under non stress conditions whearas under water stress condition it was significantly higher in C 306. Water stress significantly increased the soluble sugar content in two wheat genotypes at all the time of observations.

The genotypic variability, irrespective of treatments, was found significant only at tillering stage. However, HD 2329 exhibited higher sugar content under non stress condition. The percent increase in soluble sugar content was more in C-306 than HD-2329 under water stress condition (Table 4.29).

**Proline content**

The proline content of two wheat genotypes measured in flag leaf at tillering, anthesis and post anthesis stages have been presented in table 4.30. Data show that maximum proline content was obserbed at post anthesis stage in both the genotypes.

Proline content increased significantly on account of water stress. Genotype HD 2329 showed maximum proline content under non stress conditions while under water stress conditions higher proline content was reported in C-306. The percent increase in proline content on account of water stress was more in C-306 than HD 2329.

The genotypic differences, irrespective of the treatment, was found non significant during both the experimental years. The trend in its content was similar in both the years (Table 4.30).

## Growth, yield and Yield attributes

**Plant height**

Significant difference on plant height at maturity was observed between two genotypes during both the experimental years. The plant height was observed higher in C-306 (83.33and 88.67 cm) as compared with HD 2329 (77.87 and 80.97) in the year 2001-02 and 2003-03, respectively (Table 4.31).

Effect of water stress on the plant height was significant. It decreased from 80.60 cm to 56.38 cm in 2001-02 and 84.32to 56.68 cm in 2002-03, respectively.

Genotype x stress interaction revealed a significant reduction in plant height on account of water stress. C-306 exhibited comparatively higher plant height under both non stress and water stress conditions (Table 4.31).

**Number of effective tillers**

Non significant difference in number of effective tillers per plant at maturity stage has been observed under water stress conditions in both the genotypes. The higher numbers of tillers were recorded in HD 2329 under both non stress and water stress conditions (Table 4.31).

Genotypic variation, irrespective of stress conditions, showed that HD 2329 exhibited higher number of tillers per plant (4.33 in 2001-02 and 4.66 in 2002-03) than C-306 (3.67 in 2001-02 and 4.00 in 2002-03). However, the difference was not significant (Table 4.31)..

**Number of leaves**

A reference of data presented in table 4.32 show the number of leaves per plant recorded in two wheat genotypes during the year 2001-02 and 2002-03. A significant reduction in number of leaves on account of water stress has been observed in both the genotypes in both the years. Pooled means also reflect the similar findings.

The genotypic variations, irrespective of the water stress treatment, was found significant with higher number of leaves in HD 2329 in the year 2001-02 as well as 2002-03. The effect of water stress, irrespective of genotypes, was also found significant in both the years (Table 4.32).

**Leaf area**

There was significant difference in leaf area per plant of two wheat genotypes taken at anthesis stage. Data on genotype x stress interaction revealed that leaf area was higher in HD 2329 than C-306 in the year 2001-02 as well as 2002-03. The similar results have also obtained in pooled analysis (Table 4.32).

Highly significant decrease in leaf area was observed on account of water stress in both the genotypes. Genotypic variation, irrespective of treatments, was found significant with higher values in HD 2329 at both the years. Reduction in leaf area on account of water stress, irrespective of genotypes, was also comparable in two experimental years (Table 4.32).

**Number of spikelets**

Number of spikelets per ear recorded during 2001-02 and 2002-03 have been depicted in table 4.33. Reduction in spikelet numbers on account of water stress was found significant in both the genotypes. However, higher numbers of spikelet per ear

were recorded in HD 2329. The trend was similar in both the years as well as in pooled analysis.

Genotypic variations, irrespective of treatments, was found non significant. Water stress, irrespective of genotypes, significantly reduced the number of spikelet in both the years of experimentation (Table 4.33).

**Grain number**

Data recorded on number of grains per ear in both the years have been depicted in the table 4.33. It indicate that grain number were higher in HD 2329 under non stress as well as water stress conditions. However, the difference was significant only under non stress conditions during both the years.

Genotypic variation, irrespective of the treatments, was found significant with a higher number of grains in HD 2329. Reduction in this parameter on account of water stress, irrespective of the genotypes was also found significant (Table 4.33).

**Grain yield**

The effect of water stress on grain yield of wheat in the year 2001-02 and 2002-03 has been shown in the table 4.34. Water stress decreased the grain yield significantly in both the genotypes during both the years. The grain yield was higher in HD 2329 under non stress conditions whereas under water stress conditions, it was higher in C-306. almost similar findings were observed in both the years as well as in pooled means.

Grain yield of HD 2329, irrespective of treatments, was significantly higher over C-306 in both the years. The reduction in grain yield on account of water stress, irrespective of genotypes, was higher in the year 2001-02 as compared to 2002-03 (Table 4.34).

***Biological yield***

Data on biological yield per plant observed during 2001-02 and 2002-03 have been depicted in table 4.34. Biological yield was found significantly higher in HD

2329 under non stress conditions whereas under water stress conditions, it was higher in C-306.

Genotypic variations, irrespective treatment, was marginal but statistically significant. The reduction in biological yield under water stress conditions, irrespective of genotypes, was also significant in both the years. However, the percent reduction was higher in 2002-03 (Table 4.34).

**Test weight**

The test weight of C-306 was significantly higher over HD 2329 under water stress as well as non stress conditions in both the years. Pooled analysis also exhibited the similar trend (Table 4.35).

The C-306, irrespective of treatments, showed higher test weight in both the years. Similarly, reduction in test weight on account of water stress was found *at par* during both the years of experimentation (Table 4.35).

**Harvest index**

Data on harvest index calculated from grain and biological yields have been depicted in table 4.35. It was noted that water stress significantly reduced the harvest index during 2001-02 as well as 2002-03.

Genotypic variation, irrespective of treatments, was found significant during year2001-02. The effect of water stress, irrespective of genotypes, was also observed significant in both the years. However, the percent reduction in this parameter was higher during the year 2002-03 (Table 4.35).

**EXPERIMENT NO. 5.**

**Evaluation of the Field Efficacy of Putrescine Application in Wheat**

**Plant height**

Data on effect of putrescine on plant height at maturity in wheat genotype HD 2329 have been shown in table 4.36. It indicate that changes in plant height on account of putrescine application was non significant in the year 2001-02 and significant in the year 2002-03. The effect of putrescine concentration and mode of its application was also found non significant. The pooled analysis also showed the significant effect (Table 4.36).

***Leaf area***

A reference to data presented in table 4.36 revealed the changes in leaf area per plant at anthesis with putrescine application. The effect of putrescine concentration, irrespective of the mode of application, was found significant. However, it was increased significantly only upto 0.1 mM putrescine.

Mode of application, irrespective of concentrations, also affected the leaf area significantly. The maximum leaf area per plant was noted with two foliar sprays of putrescine.

The effect of putrescine between control and rest of the treatments was found significant in the year 2001-02 as well as 2002-03 (Table 4.36).

**Number of ears**

Result on number of ears observed in per meter row has been depicted in table 4.37. The number of ear increased significantly with all the treatments under non stress as well as water stress conditions during both the years. However, maximum increase was observed with 1.0 mM putrescine with two foliar applications. Among mode of applications, two foliar sprays have been found the best during both the years.

Data on different putrescine concentration irrespective of the mode of application also showed significant differences. This effect was found non significant after 0.1 mM putrescine (Table 4.37).

**No. of spikelets**

Number of spikelets per ear has been enhanced non significantly in the year 2001-02 as well as 2002-03 expression water stress conditions of 200-02. The pooled analysis also showed the same trend (Table 4.37).

The effect of putrescine, irrespective of mode of application, was also observed non significant. Similarly, the mode of application, irrespective of the plutrescine concentration, did non change the number of spikelets per ear significantly (Table 4.37).

**Grain number**

Effect of putrescine concentration and its mode of application on number of grains per ear have been shown in the table 4.38. Perusal of data showed that grain number increased with putrescine application but the concentration effect was non significant. Similarly, mode of application also enhanced the grain number significantly. The maximum number of grains per ear has been noticed with two foliar sprays of putrescine.

The effect of putrescine between control and rest of the treatments was found generally significant in the year 2001-02 and 2002-03 as well as in pooled analysis (Table 4.38).

**Grain weight**

The grain weight per ear has influenced significantly with putrescine concentrations as well as its mode of application (Table 4.38).

A continuous and significant enhancement in grain weight with putrescine concentrations, irrespective of the mode of application, has been observed. However, this enhancement was significant only upto 0.1 mM putrescine under water stress as well as non stress conditions.

Mode of application, irrespective of putrescine concentration, showed a significant increase in grain weight with seed treatment, which further enhanced significantly with foliar spray. Maximum increase in grain weight per ear was

recorded with two foliar sprays of putrescine but significant difference over one spray was observed only under non stress conditions (Table 4.38).

A significant increase was observed between control and rest of the putrescine treatments in both the years as well as in pooled analysis (Table 4.38).

**Grain yield**

A reference to data presented in table 4.39 show the effect of putrescine concentration and mode of application in grain yield during the year 2001-02 and 2002-03. Grain yield of wheat increased significantly with putrescine treatment under non stress and water stress conditions in both the years. However, significant effect was observed upto 0.1 mM putrescine concentrations.

Mode of application, irrespective of putrescine concentrations, also affected the grain yield significantly. Maximum enhancement in grain yield was recorded with two foliar sprays of putrescine.

A significant increase in grain yield was also observed between control and rest of the putrescine treatments in both the years as well as in pooled analysis (Table 4.39).

**Biological yield**

Data on biological yield depicted in table 4.39 also showed almost similar trend of grain yield. In general, the putrecine concentration, irrespective of the mode of application, enhanced the biological yield significantly during both the years.

Increase in biological yield with mode of application, irrespective of treatments, was also found significant with seed treatment, one foliar spray as well as with two foliar sprays (Table 4.39).

Putrescine application increased the biological yield significantly when compared with control dueing both the years under non stress as well as water stress conditions (Table 4.39).

**Test weight**

Data on effect of putrescine on test weight (1000 seed weight) depicted in table 4.40 showed that putrescine application enhanced the test weight significantly over control in the year 2001-02 and 2002-03 (Table 4.40).

Increase in test weight with putrescine concentration showed significant differences upto 0.1 mM putrescine. Mode of application also increased the test weight significantly. The maximum increase was recorded with two foliar sprays of putrescine (Table 4.40).

**Harvest index**

The changes in harvest index sown in table 4.40 has been found non significant on account of putrescine application.

The putrescine concentration as well as its mode of application irrespective of the each other, have also been found to vary non significantly during both the years (Table 4.40).

## *Chapter 5.*

# Discussion

It has been observed that photosynthetic $CO_2$ assimilation has been impaired on account of water saving strategies. This situation leads to extensive extra excitation energy, which is partially quenched by transfer of electrons from PS I to $O_2$ producing superoxide radicals (Mehler reaction). As a result, the stressed plants are exposed to the damage or destabilization of biomembranes especially through lipid peroxidation of thylakoid membrane (Aziz and Larcher 1988). The possibility of quenching extra excitation energy and increased activity of free radical scavenging enzymes are recently being used as alternative strategies to induce drought resistance and promoting growth under water deficit (Asada 1992). The similar multistress factors like salinity, drought, high temperature have shown the linkages with improved photo-oxidative tolerance of wheat genotypes which could be estimated through the higher level of anti-oxidants like ascorbic acid, glutathione and tocopherol (Sairam *et al*. 2000, Alscher *et al.* 2002). In addition, the changes in the level of different nutrients under waters tress conditions, especially nitrogen, potassium, magnesium and iron are also being considered significant (Roemheld 1999).

Superoxide dismutase, the family of metaloenzymes catalyses the disproportionation of superoxide radicals to molecular oxygen and $H_2O_2$. Superoxide dismutase removes superoxide and hence, decreases the risk of hydroxyl radical formation from superoxide via metal catalyzed type Haber –Weiss reaction (Arora *et al*. 2002). Plant peroxidases (oxidoreductase) are one of the most extensively studied plant proteins. These have been used as a convenient enzyme marker in genetic physiological and pathological studies. The role of peroxidase as " stress enzyme" in plants has been commonly acknowledged (Sharma 1999). Catalase activity has been

reported in peroxisomes which catalyze the scavenging of $H_2O_2$ to form oxygen and water.

In the present investigation, two wheat genotypes namely C-306 (drought tolerant) and HD-2329 (drought sensitive) have been undertaken to study their physio biochemical characteristics under water stress conditions. Differential response of genotypes to moisture stress depends on physio-biochemical parameters. Inbuilt systems of water economy in the tolerant genotype enable them to maintain vital physiological function under adverse climatic conditions to yield comparatively more (Sairam *et al.* 1994).

The results of investigation on "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)" are being discussed here in perspectives of available information.

Experiment No.1

## EFFECT OF WATER STRESS ON ANTIOXIDANT ENZYME ACTIVITY AND LIPID PEROXIDATION IN TWO WHEAT GENOTYPES.

The activities of enzymes namely superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) as well as hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) content have been observed under non stress and water stress conditions. The SOD activity of C-306 and HD 2329 increased differentially in two genotypes under water stress conditions. The 20% polyethylene glycol (PEG) enhanced SOD activity from 23 to 26 per cent in tolerant genotype (C-306) and from 12 to 18 per cent in susceptible genotype (HD 2329) in 9, 12 and 15 days old seedlings (Table 4.1). The average activity of superoxide dismutase also indicates the same trend (Fig. 1). It was also noted that even 10% PEG enhanced the SOD activity significantly, however, the response was variable in these genotypes. It is well recognized that plants have well developed defense system against reactive oxygen species and SOD constitutes the first line of defense (Alscher *et al*. 2002). In our study, the higher SOD activity of C-306 offers a biochemical trait of its relative drought tolerance over HD 2329 at seedling stage. Sairam *et al*. (1998) have also compared the pot grown wheat genotypes under water stress conditions and found that percent increase in SOD activity was maximum in tolerant genotype. Genotypic variability in SOD activity under saline conditions has also been observed in our lab (Sharma 2003). However, Saniyama and Tanida (1995) did not observe any variation in SOD activity in chilling sensitive and resistant rice genotypes.

The next step in enzymatic antioxidant defense involves $H_2O_2$ degrading enzymes such as peroxidase and catalase. The POX activity was almost double in C-306 as compared to HD 2329, particularly under water stress conditions (Table 4.1).

The percent increment in this enzyme activity under water stress conditions was also higher in C-306. The average POX activity at seedling stage shows the similar trend (Fig. 1). Increase in POX activity has also been reported under various stresses and has been linked with protection from oxidative damage, lignifications and cross linking of cell wall so as to prevent spread of infection during pathogen attack (Dalal and Khanna-Chopra 2001). It is suggested that elevated $H_2O_2$ concentration under water stress conditions could release POX from membrane structure with which it is normally associated (Zhang and Kirkham 1994).

Increase in catalase activity has been reported in wheat under water stress conditions (Baisak *et al*. 1994, Sairam *et al.* 1998). Our results indicate that catalase activity increased under water stress conditions in both the tolerant and sensitive genotypes (Fig 1). The percent enhancement in its activity was higher in HD 2329 but C-306 retained higher catalase activity under non stress and water stress conditions (Table 4.1). The CAT plays an important role in scavenging $H_2O_2$ (Takahashi *et al*. 1997). Studies on transgenic *CAT*-1 deficient tobacco plants revealed that it was essential for protection of ascorbate and glutathione pool from oxidation for maintaining the redox balance in cells (Willekens *et al.* 1997). Thus, availability of ascorbate and reduced glutathione may become rate limiting step during antioxidant defense system (Foyer *et al*. 1994, 1995).

The MDA content (a measure of lipid peroxidation) was significantly higher in HD 2329 over C-306 under water stress conditions. Water stress enhanced its content in both the genotypes. This increase was about 15-30 per cent more in plants treated with PEG 20% in 9, 12 and 15 days old seedlings. A critical evaluation of data showed that the MDA content in control plants of HD 2329 were *at par* with MDA content of C-306 treated with 20% PEG (Table 4.2; Fig 2). The rise in MDA content under stress suggests that water stress can induce membrane lipid peroxidation by means of activated oxygen species (Zhang and Kirkham 1994, Sairam *et al.* 1998). The lower values of MDA content in C-306 suggest that its seeds are equipped with an efficient free radical quenching system that offers protection at seedling stage against the oxidative stress and improves the vigour of the plant.

Accumulation of $H_2O_2$ increased under polyethylene glycol (PEG) induced water stress conditions in both the genotypes but it was significantly lower in C-306 under control as well as water stress conditions (Table 4.2; Fig 2). Choudhary and Choudhary (1985) in *Vigna spp* and Sairam *et al.* (1998) in wheat have also reported the lower $H_2O_2$ content in tolerant genotypes. The level of $H_2O_2$, a potent oxidant, reflects the damaging effects of stress since it can inactivate various enzymes and cause protein degradation (Asada and Takahashi 1987, Levine *et al.* 1990). Higher $H_2O_2$ content in 9, 12 and 15 days old seedlings of HD 2329 as compared to C-306 reflects the poor $H_2O_2$ scavenging capacity of a drought sensitive genotype.

The nutrients are directly linked with most of the plant processes. An imbalance in their absorption/translocation causes reduction in growth and yield of a crop. In present investigation, water stress conditions decreased the nitrogen content whereas the potassium content increased in both the genotypes specially under severe water stress i.e. 20% PEG (Table 4.3 & 4.4). Plants require potassium ions as an osmotic component and in the formation of starch, protein synthesis, photosynthetic

partitioning, and normal stomatal functioning and above all as an activator of a number of monovalent cations requiring enzymes (Epsteine 1972, Marschner 1996). Reduction in calcium content in 15 days old seedlings might be on account of reduced osmotic potential of the medium (Table 4.4).

Among trace elements, iron, manganese and copper have also been estimated (Table 4.5). Significant reduction in iron content was noticed only in 12 and 15 days old seedlings treated with 20% PEG. In case of manganese, reduction in its contents was noticed only in HD 2329 with 20% PEG treatment. The copper content was found to increase significantly under water stress conditions (Table 4.5). The higher iron content in C-306 than that of HD 2329 under water stress conditions can be linked with its role in chlorophyll synthesis and $CO_2$ assimilation. Iron is also a constituent of catalase and its higher amount in C-306 have a bearing with catalase activities for preventing the accumulation of $H_2O_2$ as discussed earlier (Table 4.1).

In nutshell, the tolerant genotype C–306 exhibited lower accumulation of MDA and $H_2O_2$ owing to increased activity of antioxidative enzymes viz. SOD, POX and CAT under PEG induced water stress conditions. Among nutrients, the status of iron and copper corroborated with tolerance and sensitivity towards stress as these cations were higher in C-306 than HD 2329.

## *Experiment No. 2*

## *ANTIOXIDATIVE ENZYME SYSTEM AND LIPID PEROXIDATION IN RELATION TO PUTRESCINE INDUCED DROUGHT TOLERANCE IN WHEAT*

The aliphatic polyamines (putrescine, spermine and sperimidine), recently recognized as a new class of plant growth substances, have been suggested to act as modulators of certain cellular and physiological processes during stress conditions (Bouchereaue *et al.* 1999, Chattopadhyaya *et al*. 2002). As already discussed, superoxide dismutase is associated with scavenging of superoxide radicals produced under moisture stress conditions (Dhindsa *et al*. 1981, Pastori and Trippi 1993, Sairam and Srivastava 2001). In present investigation, the superoxide dismutase (SOD) activity increased under water stress conditions to 31.63 and 20.89 per cent, respectively, in C-306 and HD 2329. Application of putrescine did not show pronounced effect under control conditions, however, under water stress conditions, it enhanced the SOD activity in both the genotypes with higher magnitude of enzyme activity in C-306 (Table 4.6; Fig 3). The SOD is a key enzyme in the detoxification of superoxide radicals. The increased SOD activity after putrescine treatment suggests that putrescine promoted activation of SOD might have decreased the possible toxic concentration of $O_2^-$ radicals (Zhang and Kirkham 1996, Alscher *et al*. 2002). The lower SOD activity in putrescine applied seedlings of HD 2329 than C-306 under water stress conditions suggests that it could impair the $O_2^-$ scavenging system of cells and favours accumulation of $O_2^-$ (Table 4.6). Singh *et al*. (1999) reported that

application of benzyladenine and ascorbic acid enhanced the SOD activity in senna. Brassinosteroid mediated enhanced superoxide dismutase activities have also been noticed in seedlings of tomato under high temperature conditions (Mazorra *et al*. 2002). Information on effect of putrescine on superoxide dismutase is lacking. However, in our previous studies, the physiological effects of putrescine have been found to be comparable with benzyladenine (Gupta *et al.* 2003b).

The peroxidase activity has been found to increase under polyethylene glycol (PEG) induced water stress conditions in both the genotypes. Application of putrescine enhanced the peroxidase activity in 9, 12 and 15 days old seedlings of both wheat genotypes. Average value of peroxidase activity also indicate the similar trend (Fig. 3). However, this enhancement was non significant in HD 2329 (Table 4.6). Peroxidase is an important $H_2O_2$ scavenging enzyme which is considered to remove $H_2O_2$ by oxidation of co-substrates. Kapchina and Foudouli (1991) have reported that polyamines under NaCl stress increased peroxidase activity in 11 days old pea shoots. Further, the effect was stronger with putrescine than that of spermine while spermidine caused its decline. A decreased peroxidase activity under irrigated conditions in groundnut (Vardhini and Rao 2000) and enhanced peroxidase activity in tomato seedlings under high temperature conditions (Mazorra *et al.* 2002) have also been observed. The role of polyamines in scavenging oxidative radicals has been reported in wheat (Xu *et al.* 1995). In our investigation, putrescine also decreased the lipid peroxidation in wheat seedlings (Table 4.6). It is suggested that the reduction in lipid peroxidation might be due to the enhanced activities of SOD, POX and CAT during water stress conditions (Table 4.6; Fig 3).

The exogenous application of putrescine resulted in increased catalase activity. A perusal of data further showed that increase in catalase activity on account of putrescine was non significant under control conditions whereas under water stress conditions about 2-fold increase in its activity has been noticed. Between two genotypes, C-306 always exhibited a significantly higher catalase activity in all the observations (Table 4.6; Fig 3). This increase in catalase activity might be important with SOD in eliminating $H_2O_2$ produced during stress conditions (Mazzorra *et al.* 2002). Higher catalase activity in C-306 alongwith higher activities of SOD and POX reflects that the mechanism of putrescine induced drought tolerance is operating in this genotype more efficiently. Increased catalase activity by brassinosteroids has also been reported in groundnut (Vardhini and Rao 2000).

The MDA content is expressed on a protein basis because the greater part of the lipids in wheat leaves is in the form of membranes; particularly those associated with chloroplasts and chloroplast proteins (Price and Hendry 1991). In present investigation, the $H_2O_2$ and MDA content were higher in HD 2329. Water stress conditions enhanced their contents significantly in both the genotypes (Table 4.7). Putrescine application reduced the $H_2O_2$ and MDA content. The effect was significantly perceptible under water stress conditions only (Table 4.7; Fig 4). The enhanced MDA content under water stress conditions suggests that water stress can induce membrane lipid peroxidation by means of activated oxygen species (Price and Hendry 1991, Zhang and Kirkham 1994, Sairam and Srivastava 2001).

The $H_2O_2$ is a toxic compound produced as a result of the dismutation of the superoxide radicals, and a higher concentration is injurious to the cells and the plant, resulting in lipid peroxidation and membrane injury (Pastori and Trippi 1992, Menconi *et al*. 1995). Reduction in $H_2O_2$ and MDA content by putrescine in C-306 as well as in HD 2329 under water stress conditions revealed the existence of putrescine enhanced antioxidative defense mechanism in both the genotypes but comparatively higher activities of these enzymes in C-306 might have made it more drought tolerant at seedling stage (Fig 4). Increased lipid peroxidation as a result of oxidative stress and consequently cell membrane injury have been reported (Sairam and Srivastava 2001).

It has been hypothesized that putrescine may act as protectant by altering ion concentration through membrane stabilization due to their polycationic nature. Hence, putrescine induced changes in some of the endogenous macro (N, P, K and Ca) and micro (Fe, Mn and Cu) nutrients content have been analysed under non stress and water stress conditions. The nitrogen content decreased significantly under PEG induced water stress conditions in both the genotypes. Interestingly, putrescine enhanced the accumulation of nitrogen, particularly under water stress conditions (Table 4.8). The accumulation of phosphorus also increased by putrescine under both the conditions but statistically it was non significant (Table 4.8). It indicates that putrescine also act as nitrogen source for the plant growth under water stress conditions. Increased chlorophyll contents, organic nitrogen and protein by putrescine treatment have been reported in mustard seedlings (Mishra and Sharma 1994). Effect of plant growth regulators (gibberellins and cytokinins) in ameliorating the salinity via enhanced nitrogen content and NR activity has also been observed (Angrish *et al*. 2001).

The potassium content increased and calcium content decreased under water stress conditions. Putrescine application enhanced both the nutrients but increase in calcium content was marginal and non significant in most cases. The pattern was similar in two genotypes but C-306 always exhibited higher potassium content over HD 2329 (Table 4.9). Smith (1985) suggested that putrescine contributed to maintain cellular cation-anion balance and help in stabilization of membrane integrity through their low molecular mass and polycationic nature. Tiburcio *et al.* (1994) also observed polyamines mediated stabilization of membrane damage during stress. Higher potassium level of C-306 in present investigation revealed its superiority owing to its role in osmoregulation and other physiological processes. Putrescine mediated enhancement in chlorophyll content and transpiration rate has been observed in our previous studies (Gupta *et al.* 2003b).

Among micronutrients, the manganese content remained unaltered by putrescine under non stress and water stress conditions. The accumulation of iron and copper increased in both the genotypes. However, it was non significant in most of the observations (Table 4.10). Literature on putrescine induced micronutrient accumulation is meager. The enhancement in SOD and CAT activities with concomitant increase in copper and iron leads to understand that production of highly active hydroxyl radials ($OH^-$) under stress conditions might have catalyzed by transitional metals (such as iron and copper) in Haber-Weiss cycle (Esltner 1987,

Smirnoff 1993). This hydroxyl radical generally is held to be the most likely form of activated oxygen to initiate the peroxidative destruction of lipids with consequent membrane damage (Zhang and Kirkham 1994).

In brief, it has been observed that putrescine enhanced cellular antioxidative defense mechanism under water stress conditions in both the genotypes. However, the response was more perceptible in tolerant genotype.

## EXPERIMENT NO. 3:

### Physiological Attributes of Drought Tolerance in Wheat Seedlings

Germination percentage decreased significantly under PEG induced water stress conditions in wheat genotypes C-306 and HD 2329 at 24, 48 and 72 hours. As expected, the germination percentage decreased with increasing PEG concentration (Table 4.11). The C-306 showed better germination than HD 2329 under control as well as PEG induced water stress conditions. Genotypic difference, irrespective of the treatments, was non significant (Table 4.11). Salinity and moisture stresses showed damaging impact on germination and seedling growth (Schmidhalter and Oertli 1991). Reduction in germination may be due to the less availability of free water to the seed during early hours of imbibitions thus leaving the hydrolytic enzyme inactive (Jha and Singh 1997). Inhibition of germination at high PEG concentration might be attributed to moisture deficit in the seed below the threshold requirement for germination. Therefore, it seems possible that PEG exert major influence on seed germination by retarding water imbibitions (Everiff 1983).

A significant decrease in root and shoot length was also observed in 5, 7, 9, 12 and 15 days old seedlings in both the genotypes. Percent reduction in these parameter was maximum at 20% PEG. However, the drought tolerant genotype C-306 showed higher growth than drought sensitive genotype HD 2329 (Table 4.12 & 4.13). Effect of PEG induced water stress on reduced growth of root and coleoptiles can be explained by reduction in absorption of moisture by endosperm and embryonic axis and delayed translocation of carbohydrates to embryonic axis as suggested by Schmidhatler and Oertli 1991). Reduced seedling growth may also be due to the inhibition of processes such as cell division, enlargement and differentiation associated with water deficit. The present investigation revealed the decrease in seedling vigour index under PEG induced water stress in both the genotypes. Genotypic differences, irrespective of the treatment, were also found significant (Table 4.14; Fig 5). Overall, the drought tolerant genotype showed better performance compared with susceptible one.

Data on relative water content measured in 5, 7, 9, 12 and 15 days old seedlings revealed its significant decline under PEG induced water stress conditions. Between the two genotypes tested, C-306 maintained higher relative water content under water stress conditions compared to HD 2329. Genotypic differences, irrespective of the treatment, were also found significant. The C-306 retained more water in the seedlings compared to the drought susceptible genotype HD 2329 (Table 4.15; Fig 5). Retention of more water in seedlings is known to be important to

drought tolerance (Morgan 1983, El *et al.* 1998). Rekika *et al*. (1998) reported that higher relative water content could help the tolerant genotype to perform various physiological and biochemical processes more efficiently under water stress conditions than susceptible genotypes.

Membrane stability index decreased significantly on account of PEG induced water stress in 5, 7, 9, 12 and 15 days old seedling. Genotypic variations were also significant. Higher index was observed in C-306 both under control as well as PEG induced water stress (Table 4.16; Fig 6). The lower membrane stability reflects the extent of lipid peroxidation (Dhindsa *et al*. 1981), which in turn is a consequence of higher oxidative stress due to various environmental stresses (Leibler *et al*. 1986). These results strengthens our findings (Experiments 1 and 2) where higher lipid peroxidation in HD 2329 than drought tolerant genotype C-306 has been observed (Table 4.2 & 4.7). Lower lipid peroxidation in C-306 might be due to high activity of SOD, CAT and POX which scavenge harmful radical such as $O_2^-$ and superoxide protect the cell membrane from these oxidative stresses (Sairam and Saxena 2000, Aziz and Larcher 1998).

Soluble sugars, proline, betaine etc. are important plant osmolytes which are reported to accumulate under water stress conditions (Gupta *et al*. 2001). In present investigation, the total sugars followed a trend of increase with increasing level of moisture stress (Table 4.17). The percent increase was more in tolerant genotype C-306 than susceptible genotype HD 2329. Further, the tolerant genotype maintained higher sugar content than susceptible one in all the observations (Table 4.17). The accumulation of more sugars in tolerant genotye suggests a protective function through osmoregulation by decreasing osmotic potential of a cell and maintaining a positive turgor (Jha and Singh 1997).

Water stress causes a marked change in protein synthesizing apparatus of plant tissue and the capacity for protein synthesis also decreased considerably in response to water stress (Gupta *et al*. 2001). Our results indicate that reduction in protein content on account of PEG induced water stress was higher in C-306. Its content diminished continuously with increasing PEG concentration in both the genotypes. Tolerant genotype had comparatively higher protein content compared to susceptible during PEG induced water stress in 5, 7, 9, 12 and 15 days old seedlings (Table 4.18). Garg (2000) reported that higher protein content in tolerant genotype under water stress could be attributed to its high DNA and RNA content, which stimulate its synthesis and inhibits its decomposition.

A significant decrease in total chlorophyll content was observed under water stress conditions in 5, 7, 9, 12 and 15 days old seedlings. The C-306, a drought tolerant genotype, showed lesser reduction in chlorophyll content than HD 2329 (Table 4.19). Chlorophyll content temporarily increased with advancement of day. Higher chlorophyll content of C-306 reflects its relative tolerance to water stress (Fig. 6). It has been reported that chlorophyll content is directly linked with photosynthetic efficiency and carotenoids are responsible for scavenging of singlet oxygen and hence, their comparative levels in a genotype can determine its relative tolerance (Knox and Dodge 1985).

These findings suggests that PEG induced water stress conditions reduced the seedling growth and physiological processes at seedling stage in both the genotypes. However, the adverse effect of water stress was more pronounced in HD 2329 than C-306 in most of the observations.

**EXPERIMENT NO. 4.:**

**Physiological Attributes of Drought Tolerance in Wheat Genotypes**

Wheat crop responds to water deficit in the form of changes in various physiological and biochemical processes. In present investigation, the water stress conditions were created by withholding irrigation at tillering, anthesis and post anthesis stages and various physiological and biochemical observations were recorded. The reduction in photosynthetic rate in wheat crop as a result of water stress has been reported (Xue *et al*. 1992, Rekika *et al*. 1998). In our investigation, the reduction in rate of photosynthesis was maximum at anthesis stage in both the years of experiment as well as in pooled analysis. However the level of reduction in drought tolerant genotype C-306 was less as compared to susceptible genotype HD 2329 (Table 4.20; Fig 7). Further, C-306 showed lesser reduction in photosynthetic rate on account of water stress and at the same time maintained higher photosynthetic rate under water stress conditions than HD 2329. However, under non stress conditions HD 2329 showed higher photosynthetic rate (Table 4.20; Fig 7).

The rate of photosynthesis depends on stomatal and non stomatal components and each of the component has a unique response to an environmental variations (Bethke and Drew 1992). The stomatal conductance is related to turgor pressure of cell. The turgor pressure is controlled by solute regulation within guard cells and the relative water content of epidermal tissues. Water stress altered the osmotic activity, causing a reduction in water potential and ultimately leading to stomatal closure due to loss of guard cell turgor (Hasegawa *et al.* 2000, Lidon and Teixeira 2000). A higher internal $CO_2$ concentration in HD 2329 than C-306 indicates that the main cause of its lower photosynthesis is the photosynthetic capacity of mesophyll cells rater than its lower stomatal conductance (Table 4.22 & 4.23). Xue *et al.* (1992) reported a decrease in RUBISCO activity under water stress and this enzyme activity was affected earlier and to a great extent in susceptible than tolerant cultivars.

The present investigation revealed the reduction in transpiration rate and stomatal conductance and increase in internal $CO_2$ as a result of water stress (Table 4.21, 4.22 & 4.23; Fig 8) in both the years of experiment as well as in pooled analysis. The HD 2329 registered higher rate of transpiration and stomatal conductance under non stress conditions but under water stress condition the rate of transpiration was higher in C-306. Siddique *et al.* (1999) reported that exposure of plants to drought stress led to a significant decrease in photosynthetic rate and stomatal conductance and concomitant increase in intercellular $CO_2$ concentration. Kumar and Sankhla (1983) also observed that stomata of desert plants respond to soil moisture and ambient humidity. In the present investigation, the rate of transpiration decreased with the advancement of days to maturity (Table 4.21; Fig 7). It might be due to the cumulative effect of decrease in soil moisture content. The stomatal behaviour, which

influences the water status of the plant tissue during the period of water deficit, is of importance in determining the growth rate of plants during drought (Yadava *et al.* 1994). The present investigation revealed variation in stomatal conductance and internal $CO_2$ concentration between two genotypes under water stress conditions (Table 4.22 & 4.23). Significant genotypic variations under water stress conditions have been reported in leaf conductance (Fischer and Sanchez 1979, Gupta *et al*. 2001). Singh *et al.* (1994) reported high ratio of abaxial to adaxial transpiration as a measure of drought tolerance in wheat genotypes. Decrease in transpiration rate (Kumar and Gour 1992, Strauss and Agenbeg 2000) and increase in leaf temperature (Wang *et al.* 1993, Ravichandran and Munsge 1999)) have been reported as the measure of drought tolerance in wheat genotypes. The present investigation revealed decrease in leaf transpiration rate, and stomatal conductance in water stressed plants in both the genotypes (Table 4.21 & 4.22; Fig 7 & Fig 8).

The water use efficiency is the ratio of photosynthesis to transpiration. In present investigation, C-306 showed higher water use efficiency than HD 2329. Maximum water use efficiency was reported at anthesis stage both under non stress and water tress conditions (Table 4.26). It might be due to higher rate of photosynthesis and lower transpiration rate at anthesis. Massacci *et al.* (1996) reported that closure of stomata and decline in photosynthesis have been accompanied by slightly reduced water use efficiency under water stress. Kumar *et al*. (1993) observed high interruption of photo synthetically active radiation by drought tolerant genotypes as compared to sensitive one under water stress conditions.

Membrane stability index (MSI) was higher in C-306 compared to HD 2329 under both non stress and water stress conditions. It decreased significantly on account of water stress. Further, the percent reduction in MSI was more in HD 2329 (Table 4.24; Fig 9). The lower MSI reflects the effect on lipid peroxidation (Dhindsa *et al*. 1981), which in turn is a consequence of higher oxidative stress due to various environmental stresses (Leibler *et al*. 1986). It has also been reported that cell membrane stability is an indicator of drought tolerance (Premchandra *et al*. 1990, Gupta *et al*. 2000). Lower membrane stability has also been reported in susceptible genotypes of wheat (Sairam *et al*. 1998), maize (Pastori and Trippi 1992) and groundnut (Deb *et al*. 1996).

It has been reported that under water stress conditions, the relative water content decreased, but the percent reduction was lesser in tolerant varieties than the susceptible varieties (Richie *et al.* 1990, Kumar *et al.* 1994, Martein *et al*. 1997). In present investigation, drought tolerant genotype C-306 exhibited higher relative water content both under non stress and water stress condition. The reduction in relative water content on account of water stress was higher in HD 2329 as compared to C-306 (Table 4.25; Fig 9). Islam *et al*. (1998) reported that percent reduction in relative water content was relatively rapid in drought susceptible genotypes of wheat.

Gupta *et al*. (2000) and Sairam and Saxena (2000) reported that water stress reduced chlorophyll content but its reduction in drought tolerant wheat genotypes was at minimal level than drought susceptible genotypes. Our results showed that the drought tolerant genotype C-306 exhibited higher chlorophyll and carotenoid content than HD 2329 under water stress conditions (Table 4.27, 4.28;). The table also

indicates higher reduction in chlorophyll and carotenoid content in HD 2329 than C-306 under water stress. It might be attributed to increased activity of chlorophyll degrading enzyme chlorophyllase (Reddy and Vora 1986). Carotenes are responsible for scavenging of singlet oxygen (Knox and Dodge 1985) and hence, their comparative level in a genotype can determine its relative tolerance. Higher chlorophyll and carotenoid content in tolerant wheat genotypes have also been reported earlier (Sairam 1994, Kraus 1995).

Many workers reported accumulation of osmotically active metabolites such as proline, amino acids, soluble sugars and polyamines under water stress conditions. Khan *et al*. (1999) reported that osmotic adjustment by accumulation of metabolites and maintenance of turgor by decreasing osmotic potential at lower leaf water potential are the mechanism of drought tolerance in wheat genotypes. The present investigation showed an increased accumulation of proline and soluble sugar under water stress conditions in both the genotypes (Table 4.29 & 4.30). Our data further showed that the extent of accumulation of such metabolites was higher in drought tolerant genotype C-306 as compared to HD 2329 except at tillering stage. Kashem *et al*. (2000) reported that plant synthesize sucrose to prevent the important organelles of the cells under stress conditions. Blum *et al*. (1999) concluded that the consistent genotypic difference in osmotic adjustment exists among wheat cultivars and those with high osmotic adjustment cultivars tended to yield better than lower osmotic adjustment cultivars under preflowering drought.

Decrease in number of grains per spike (Wang *et al.* 1993), spike length (Jamal *et al*. 1996), number of spikes and tiller number (Jat *et al*. 1990), plant height and grain weight (Jat *et al*. 1990. Saha and Paul 1997), shoot dry matter production (Klar *et al.* 1990) and leaf area (Gupta *et al.* 2001) were reported on account of water stress. Our results indicate that reduction in most of the growth attributes was significant in both the genotypes. However, percent reduction was higher in HD 2329 than C-306 (Table 4.31, 4.32 & 4.33). It is suggested that reduction in grain yield on account of water stress might be through inhibition of photo assimilation at early stages because water stress reduces the content of photosynthetic pigments and sugars in the leaves. These changes might cause the decline in leaf photosynthesis leading to the poor sugar accumulation in grains (Sulatana *et al*. 1999). The investigation further revealed lower reduction in most growth and yield components in the drought tolerant genotype C-306 as compared to HD 2329 (Table 4.34 & 4.35). Drought tolerant genotypes also show recovery in growth after the removal of stress (Sarkar 1994). The reduction in leaf area per plant, which has considerable effect on grain yield on account of water stress has also been reported (Jat and Kumar 1994, Gupta *et al.* 2003b). Our results depicted in the table 4.32 show that both number of leaves and leaf area have been significantly affected on account of water stress. The genotypes showed variable response. The reduction in leaf area was lower in drought tolerant genotype C-306 than HD 2329 (Table 4.32). Karim *et al*. (2000) observed reduction in effective tillers on account of water stress at heading stage. The results showed that although HD 2329 showed higher number of productive tiller under non stress condition but reduction was lower in C-306 under water stress conditions (Table 4.31). Grain number has been found as one of the most important yield attributes

under water limiting conditions (Jat *et al*. 1991, Jamal *et al*. 1996). In present investigation, HD 2329 showed higher grain number than C-306 under non stress conditions but percent reduction on account of water stress was higher in HD 2329 (Table 4.33).

The anthesis stage has been reported to be most sensitive stage to water stress in wheat (Jamal *et al*. 1996, Ravichandran and Mungse 1997, Gupta *et al.* 2001). The present results showed significant reduction in grain yield on account of water stress imposed at tillering, anthesis and post anthesis stages (Table 4.34). The data also shows reduction in biological yield on account of drought in the period between stem elongation and heading. In general, grain and biological yield were higher in HD 2329 under non strsssed conditions whereas C-306 showed higher yields under water stressed conditions (Table 4.34). Data depicted in table 4.35 show reduction in harvest index and 1000 seed weight on account of water stress. It further indicates that the reduction in grain yield was to greater extent than reduction in biological yield on account of water stress. Drought tolerant genotype C-306 showed higher harvest index under water stress conditions (Table 4.35). Reduction of harvest index on account of water stress (Moreira *et al*. 1999, Gupta *et al*. 2003b) and its usefulness as criterion to assess drought tolerant in wheat has been reported (Stankova 1998).

It is suggested that higher yield of HD 2329 under non stress conditions might be on account of its higher yield potential. The C-306 exhibited higher yield owing to the existence of drought tolerance mechanism at physio-biochemical levels in terms of gas exchange, stomatal regulation, membrane permeability, photosynthetic pigments and osmotically active metabolites at critical growth stages of wheat.

**EXPERIMENT NO. 5**

**Evaluation of the Field Efficacy of Putrescine Application in Wheat**

Effects of exogenously applied polyamines on physiology, growth and yield attributes have been documented in literature (Slocum and Flores 1991, Sharma and Ali, 1988, Chattopadhyaya 2002, Gupta *et al.* 2003b). In present investigation, exogenous application of putrescine has enhanced the grain yield in wheat genotype HD 2329 (Table 4.39; Fig 10). Putrescine concentration also affected the enhancement of grain yield and it was observed maximum with 0.1 mM putrescine. At higher concentration, a non-significant increase was noted. Perusal of data further showed that the response of putrescine was more pronounced under water stress conditions. It increased the grain yield regardless of the mode of application but the effect was more pronounced with two foliar sprays of putrescine (Table 4.39; Fig. 10). In another study, the significant response of different modes of putrescine

application on grain yield has been observed. Maximum yield was observed with a combination of seed treatment plus spray of putrescine (Gupta *et al.* 2003b).

Biological yield was also enhanced by putrescine application. Its maximum values have been recorded with 1.0 mM concentration of putrescine but the effect became non significant after 0.1 mM concentration (Table 4.39). Harvest index is an important parameter to understand the source sink relationship. In our study, putrescine induced changes in harvest index has been found non significant under non stress and water stress conditions (Table 4.40). It is suggested that a concomitant increase in biological yield alongwith grain yield might have resulted in non significant variation in harvest index. It has been reported that the physiological functions of putrescine are comparable to cytokinin (Slocum and Flores, 1991; Gupta *et al*. 2003a) and the effect of cytokinin in enhancing grain and biological yield in wheat has been reported by various workers (Gabali *et al*. 1986; Warrier *et al.* 1987; Gupta *et al.* 2003a). It has been suggested that the improvement in yield of tomato by putrescine application has been attributed in endogenous polyamines remained for the normal development of reproductive structures (Cohen *et al*. 1982). However, information on effect of polyamines on the growth and productivity of wheat is almost lacking.

Grain weight and grain number are two most important yield components of wheat. A critical evaluation of the yield data in present investigation revealed that the effect of putrescine was more perceptible in grain weight as compared to grain number. The mode of application affected both grain weight and grain number significantly but the effect of putrescine concentration on grain number was found non significant (Table 4.38). It means, putrescine might have increased the grain yield by strengthening the sink efficiency via increased photosynthesis or stem reserve mobilization towards developing sink (Blum *et al*. 1994). Significant effect of mode of putrescine application on grain number suggests its role in grain setting. The role of exogenously applied cytokinin on grain weight without affecting grain number have been reported in our previous studies (Gupta *et al*. 2000). Further, the response

of cytokinin was more pronounced in younger grain of wheat than older grains (Gupta *et al*. 2003a).

The effect of exogenous application of putrescine has also been observed in other yield contributing parameters. Plant height and number of spikelets changed non significantly whereas increase in number of effective tillers and leaf area were observed significant (Table 4.36 & 4.37). Putrescine induced increase in chlorophyll content, organic nitrogen and protein content in leaf tissue have been reported in mustard (Mishra and Sharma 1994). Enhanced leaf area in present investigation might have helped in fulfilling the enhanced demand of developing sink, particularly under waters stress conditions. Liu *et al*. (2000) suggested that polyamines target KAT 1 like inward $K^+$ channel in guard cell and modulate stomatal movement, providing a link between stress conditions, polyamine levels and stomatal regulation. This property may be linked to the increased transpiration rate and stomatal conductance caused by polyamine application in wheat. In soybean, foliar spray of $10^{-3}$ M polyamines at 50 per cent flowering stage showed increasing trend on number of pod per plant, 100 seed weight, seed and oil yield (Sharma and Ali 1998). In rice, grain filling and grain weight were found positively correlated with polyamines content particularly with spermine and spermidine. Fruit set in olive also increased by spray of putrescine during flowering. Exogenous application of polyamines also increased the yield in horticultural crops such as litchi and apple (Costa and Bagni. 1983)

In present investigation, putrescine increased the yield significantly with different treatments when compared with control. The effect was noticeable under stress as well as non stress conditions. It can be noted that application of putrescine under non stress conditions boosted the productivity of wheat by 9.81 per cent whereas the corresponding figure under water stress condition was 18.5 per cent. It clearly shows that the benefit of applying putrescine was more proved under water stress conditions. This finding has valuable implication for the wheat growers of semi arid region. Further, economic analysis of data shows that albeit maximum grain yield was recorded with 0.1 mM concentration with two mode of application, the maximum net return was obtained with 0.01 mM at two mode of application (Appendix III).

This may be due to the high cost of putrescine. The cost of treatment may also be deterrent to farmers. Yet, it is an economically viable preposition can increase their profit by using putrescine. This finding calls for further research to cut down the cost of putrescine or evaluating commercial formulation of polyamines for inducing drought tolerance.

There have been the cases with other plant growth regulators also which had been very costly in the beginning but when their efficacy under field condition for crop amelioration was established, the efforts were made at industrial level to cut down the cost of the chemical and these efforts have resulted in production of economically viable agricultural formulations of several plant growth regulators.

*Chapter 6*

# Summary

Studies entitled "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)" were conducted at the Department of Plant Physiology during rabi 2001-02 and 2002-03 with the following objectives:

1. To study variation in antioxidant enzyme activity and lipid peroxidation in wheat genotypes.
2. To study variation in antioxidant enzyme activity in relation to putrescine induced drought tolerance in wheat.
3. To evaluate the field efficacy of putrescine application.

In order to achieve these objectives, following five experiments have been conducted in the laboratory, pot culture and field conditions. The details of the experiments and their salient research findings are as follows:

**Experiment No 1**

A laboratory experiment on "Effect of water stress on antioxidant enzyme activity and lipid peroxidation in contrasting wheat genotypes" have been conducted on two wheat genotypes namely C-306 (drought tolerant) and HD 2329 (drought sensitive and widely adapted). These genotypes were raised in root trainers filled with loamy sand soil in the growth chamber. Water stress conditions were created by irrigating these cells with PEG 6000 (10 and 20 %). Cells of root trainers irrigated with distilled water were served as control. Observations on antioxidative defense mechanism and nutrient content have been recorded in 9, 12 and 15 days old seedings of both the genotypes. . Salient research findings of these studies are:

1. SOD, POX and CAT activities increased significantly under PEG induced water stress conditions in 9, 12 and 15 days old seedlings of two wheat genotypes i.e. C-306 and HD 2329. Genotypic variations were significant. The

CAT and POX activities were higher in genotype C-306 under both non stress and water stress conditions.

2. MDA and $H_2O_2$ content increased significantly and linearly with increasing PEG induced water stress conditions in both the genotypes. Their contents were higher in susceptible genotype HD 2329 under control conditions, which further increased under water stress conditions. The genotypic variations were statistically significant.
3. The C-306 exhibited significantly higher nitrogen content over HD 2329 under water stress conditions. The phosphorus content decreased non significantly on account of water stress in both the genotypes.
4. The potassium content increased while calcium content decreased under water stress conditions. Genotypic variations in potassium and calcium content were also significant.
5. Water stress induced reduction in manganese content was significant only in 12 and 15 days old seedlings of HD 2329. The iron content also decreased in both the genotypes. In general, C-306 accumulated higher amount of iron under water stress and non stress conditions. Copper content generally increased non significantly under PEG induced water stress conditions.

These findings suggest that cellular antioxidative defense system exists in wheat seedlings and drought tolerant genotype C-306 showed higher antioxidative enzyme activity and lower lipid peroxidation in term of MDA than HD 2329 under PEG induced water stress conditions. Further, nutrient status is corroborated with tolerance and sensitivity of genotypes.

**Experiment No 2**

Another laboratory experiment on "Changes in antioxidant enzyme activity and lipid peroxidation in relation to putrescine induced drought tolerance in wheat" has been conducted in root trainers in the growth chamber. Seed of wheat genotypes C-306 and HD 2329 were soaked in putrescine dihydrogen chloride (0.1 mM) for six hour followed by air-drying and then sown in individual cell of the root trainers. The control plants were soaked in distilled water and sown in the same manner. Irrigating these cells with water containing 20 percent PEG-6000 created the water stress conditions. Antioxidants, lipid peroxidation and nutrient contents have been estimated in 9, 12 and 15 days old seedlings in both the genotypes. Salient research findings of these studies are:

1. Putrescine enhanced SOD, POX and CAT activities, particularly under water stress conditions. Percent increase in these enzymes under water stress conditions was higher in C-306 as compared to HD 2329.
2. The MDA and $H_2O_2$ content increased significantly under water stress conditions in both the genotypes in 9, 12 and 15 days old seedlings. Putrescine treatment reduced their contents under water stress conditions. The tolerant genotype C-306 retained lower amount of $H_2O_2$ and MDA content in all the observations.
3. Nitrogen and phosphorous content decreased significantly under water stress conditions in both the genotypes. Putrescine application increased accumulation of these nutrients, particularly under water stress conditions.
4. Potassium accumulation increased by putrescine significantly whereas enhancement in calcium accumulation was non significant. The values for potassium content were higher in C-306.
5. The iron, manganese and copper content were *at par* in two genotypes under control conditions. The iron contents decreased under water stress conditions and putrescine treatment tried to retain the levels under both the conditions. The iron and copper contents were generally higher in C-306 under PEG induced water stress conditions.

On the basis of these findings it can be inferred that putrescine tried to alleviate the effect of water stress by increasing the antioxidant enzyme activity and nutrient accumulation.

**Experiment No 3**

A laboratory experiment on "Physiological attributes of drought tolerance in wheat" has been conducted in the same wheat genotypes. Seeds of these genotypes have been sown in petriplates with 0, 10 and 20 % PEG-6000. After seven days, the seedlings of these genotypes were transferred to the root trainers filled with loamy sand soil and provided the same treatments. The germination percentage was recorded in petriplates at 24, 48 and 72 h after incubation whereas

observations on various physio-biochemical parameters have been recorded in 5, 7, 9, 12 and 15 days old seedlings. Salient research findings of these studies are

1. Germination percentage decreased significantly with PEG induced water stress conditions. Genotypic variation was found non significant.
2. Root length, shoot length and seedling vigour index reduced significantly under water stress conditions in 9, 12 and 15 days old seedlings. Drought tolerant genotype C-306 performed better over HD 2329.
3. RWC and MSI decreased significantly by PEG induced water stress conditions in 5, 7, 9, 12 and 15 days old seedlings. Genotypic variations were also significant. The C-306 exhibited higher RWC and MSI than HD 2329.
4. Sugar content enhanced linearly and significantly with increasing PEG concentration in both the genotypes. The percent increase was higher in C-306 as compared to HD 2329.
5. Reduction in chlorophyll and protein content on account of PEG induced water stress conditions was higher in HD 2329. Their contents diminished continuously with increasing PEG concentration in both the genotypes.

These findings suggest that PEG induced water stress conditions reduced the growth and physiological parameters at seedling stage of wheat genotypes. The relative responsiveness of the genotypes in terms of these parameters indicates that c-306 is more tolerant to water stress conditions than HD 2329.

**Experiment No 4**

A pot experiment on "Physiological attributes of drought tolerance in wheat" has been conducted on the same wheat genotypes (C-306 and HD 2329) in the pot culture. The water stress treatments were given at tillering, anthesis and post anthesis stages by withholding water supply for a uniform period of 8 days. Observations on various physiological attributes were recorded at these stages whereas yield and its contributing parameters were taken after harvesting the crop. Salient research findings of these studies are:

1. Photosynthesis, transpiration and stomatal conductance were reduced significantly whereas internal $CO_2$ concentration increased significantly under water stress conditions at tillering, anthesis and post anthesis stages. The significant genotypic variation was observed in internal $CO_2$ concentration. Genotypic variation in water use efficiency was significant during both the

years. Pattern of these parameters was almost similar in both the years of experimentation.

2. The RWC, MSI, chlorophyll and carotenoid content decreased significantly on account of water stress in both the genotypes at all three stages. The C-306 exhibited higher amount of these parameters than HD 2329.
3. Soluble sugar and proline increased significantly with water stress at different growth stages. The magnitude of increase was higher in C-306 than HD 2329.
4. Number of effective tillers, number of leaves, leaf area, number of spikelet per ear, number of grains per ear, grain yield, biological yield and harvest index were higher in HD 2329 under non stress conditions but under water stress conditions these parameters were observed higher in C-306.

It is concluded that comparatively higher grain yield of C-306 under water stress conditions might be due to its drought tolerance characteristics like better water use efficiency, higher RWC, MSI, accumulation of osmotically active metabolites, etc. The lower reduction in growth and yield attributes on account of water stress might contributed in better yield performance of C-306.

**Experiment No 5**

A field experiment on "Evaluation of the field efficacy of putrescine application in wheat under water stress and non stress conditions" has been conducted in rabi 2001-02 and 2002-03. Wheat genotype HD 2329 was sown in randomized block design and putrescine (0.01. 0.1, 1.0 mM concentration) were applied as seed treatment, one foliar spray or two foliar sprays. For water stress treatment, the number of irrigations has been reduced to half (three) as compared to control (six). Harvesting was done at maturity and growth and yield attributes have been worked out for both the conditions. Salient research findings of these studies are:

1. Putrescine application enhanced leaf area upto 0.1 mM concentration in both the years of experiments i.e. 2001-02 and 2002-03. In case of mode of application, maximum leaf area was recorded significantly upto one foliar spray.
2. Grain weight and grain yield enhanced significantly with putrescine concentration. However, after 0.1 mM putrescine increase in grain weight and yield were non significant. Among mode of application, one foliar

application of putrescine performed the best. Grain number also increased with putrescine.

3. Biological yield increased significantly upto 0.1 mM putrescine concentration in both the years. Significant increment in this parameter was observed with all the modes of putrescine application. Effect of putrescine on harvest index was found non significant.

It is concluded that putrescine application enhanced yield and yield contributing parameters under non stress and water stress conditions. The best results have been obtained with 0.1 mM putrescine and with two foliar applications.

## Appendix – I

## Mean weekly weather parameters during crop seasons

| No. of week | *Period* | Temperature (°C) | | | | Relative Humidity (%) | | Total rainfall (mm) | | Evaporation (mm/day) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Maximum | | Minimum | | | | | | | |
| | | 01-02 | 02-03 | 01-02 | 02-03 | 01-02 | 02-03 | 01-02 | 02-03 | 01-02 | 02-03 |
| 40 | Oct 01 to Oct 07 | 35.5 | 38.3 | 20.5 | 18.3 | 55 | 37 | - | - | 5.9 | 7.4 |
| 41 | Oct 08 to Oct 14 | 35.9 | 37.6 | 17.9 | 20.0 | 47 | 45 | - | - | 5.6 | 7.4 |
| 42 | Oct 15 to Oct 21 | 34.7 | 35.3 | 12.5 | 17.3 | 41 | 48 | - | - | 4.4 | 5.4 |
| 43 | Oct 22 to Oct 28 | 35.9 | 34.1 | 13.5 | 12.0 | 43 | 42 | - | - | 4.6 | 4.6 |
| 44 | Oct 29 to Nov 04 | 35.1 | 34.4 | 13.7 | 12.9 | 45 | 42 | - | - | 4.2 | 4.0 |
| 45 | Nov 05 to Nov 11 | 31.8 | 31.9 | 13.4 | 13.5 | 53 | 43 | - | - | 4.0 | 4.2 |
| 46 | Nov 12 to Nov 18 | 30.4 | 28.6 | 8.1 | 10.5 | 41 | 47 | - | - | 4.0 | 4.1 |
| 47 | Nov 19 to Nov 25 | 47.3 | 30.8 | 7.7 | 10.5 | 42 | 39 | - | - | 3.9 | 4.3 |
| 48 | Nov 26 to Dec 02 | 27.5 | 29.4 | 7.0 | 8.6 | 47 | 47 | - | - | 3.2 | 3.8 |
| 49 | Dec 03 to Dec 09 | 29.2 | 27.4 | 7.2 | 7.1 | 44 | 46 | - | - | 3.2 | 3.9 |
| 50 | Dec 10 to Dec 16 | 27.1 | 28.6 | 7.9 | 8.5 | 59 | 47 | - | - | 3.6 | 4.1 |
| 51 | Dec 17 to Dec 23 | 24.6 | 29.1 | 4.1 | 8.7 | 57 | 51 | - | - | 2.6 | 3.6 |
| 52 | Dec 24 to Dec 31 | 30.8 | 22.6 | 3.6 | 5.7 | 61 | 67 | - | - | 2.3 | 3.5 |
| 01 | Jan 01 to Jan 07 | 22.5 | 18.1 | 3.0 | 4.9 | 61 | 69 | - | 2.0 | 2.2 | 1.1 |
| 02 | Jan 08 to Jan 14 | 24.1 | 20.9 | 5.0 | 2.5 | 59 | 59 | - | - | 1.8 | 1.4 |
| 03 | Jan 15 to Jan 21 | 20.5 | 22.2 | 7.9 | 2.0 | 77 | 62 | 2.0 | - | 1.3 | 1.8 |
| 04 | Jan 22 to Jan 28 | 21.4 | 24.0 | 3.7 | 7.6 | 59 | 54 | - | - | 2.4 | 2.5 |
| 05 | Jan 29 to Feb 04 | 21.7 | 19.6 | 1.9 | 7.1 | 57 | 52 | - | - | 2.3 | 2.5 |
| 06 | Feb 05 to Feb 11 | 22.2 | 23.8 | 3.6 | 5.3 | 57 | 58 | - | 2.0 | 2.5 | 3.6 |
| 07 | Feb 12 to Feb 18 | 23.0 | 24.8 | 5.8 | 8.1 | 61 | 57 | - | - | 2.9 | 3.1 |
| 08 | Feb 19 to Feb 25 | 26.7 | 22.8 | 7.5 | 10.5 | 52 | 51 | - | - | 4.3 | 2.6 |
| 09 | Feb 26 to Mar 04 | 30.5 | 30.3 | 11.8 | 15.7 | 54 | 46 | - | - | 4.8 | 3.6 |
| 10 | Mar 05 to Mar 11 | 28.8 | 29.0 | 8.9 | 8.4 | 39 | 49 | - | - | 4.8 | 4.0 |
| 11 | Mar 12 to Mar 18 | 31.9 | 29.6 | 11.5 | 11.8 | 39 | 42 | - | - | 5.5 | 3.6 |
| 12 | Mar 19 to Mar 25 | 35.5 | 34.3 | 14.5 | 15.9 | 39 | 48 | - | - | 5.9 | 5.7 |
| 13 | Mar 26 to Apr 01 | 35.4 | 36.1 | 14.2 | 18.3 | 33 | 48 | - | - | 1.6 | 6.7 |
| 14 | Apr 02 to Apr 08 | 37.2 | 36.1 | 16.3 | 16.8 | 35 | 36 | - | 8.1 | 1.6 | 6.5 |
| 15 | Apr 09 to Apr 15 | 37.4 | 38.8 | 16.2 | 18.5 | 35 | 37 | 0.8 | - | 7.2 | 7.9 |

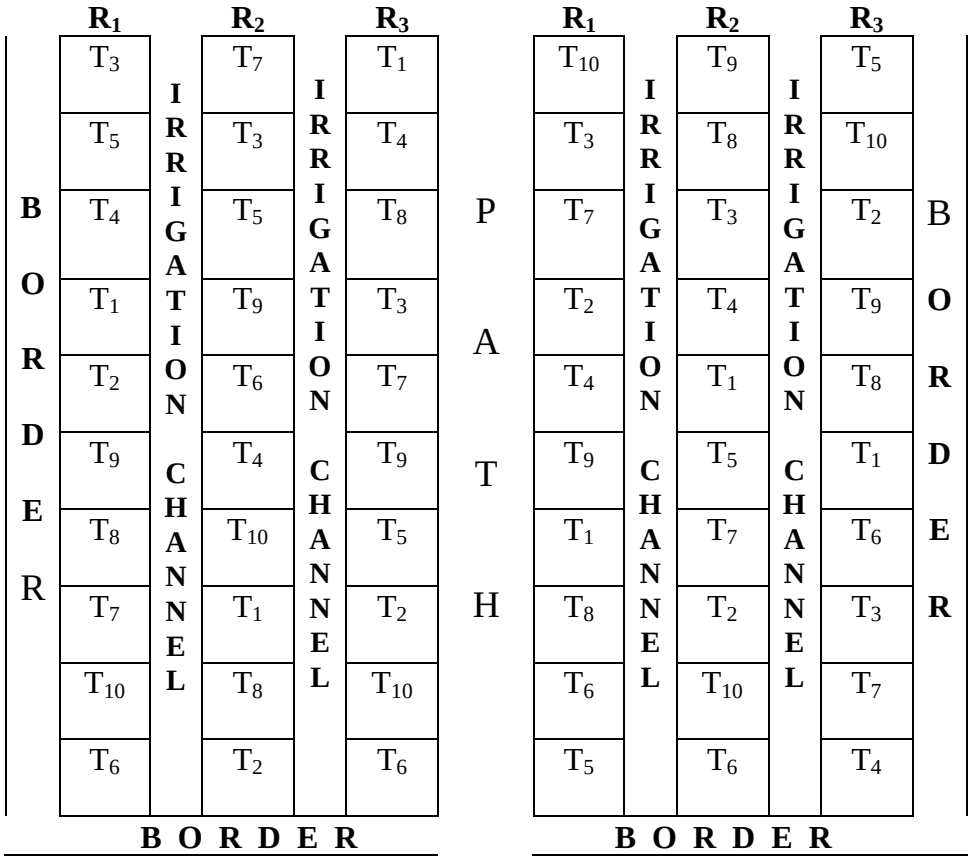
**Appendix – II**

LAYOUT PLAN OF EXPERIMENT NO. 5

Genotype: HD 2329
Condition: Non stress, water stress
Concentration: Three (0.01mM, 0.1 mM, 1.0 mM)
Mode of application: i) Seed treatment
ii) One spray (Anthesis)
iii) Two sprays (Anthesis and post anthesis)

**N**

STRESS NON STRESS




Experimental design: RBD
Replications: three
Plot size: 2 x 3 m

Treatments: Ten

| | |
|---|---|
| $T_1$ Seed treatment (1.0 mM Putrescine) | $T_6$ One spray (0.01 mM Putrescine) |
| $T_2$ Seed treatment (0.1 mM Putrescine) | $T_7$ Two spray (1.0 mM Putrescine) |
| $T_3$ Seed treatment (0.01 mM Putrescine) | $T_8$ Two spray (0.1 mM Putrescine) |
| $T_4$ One spray (1.0 mM Putrescine) | $T_9$ Two spray (0.01 mM Putrescine) |
| $T_5$ One spray (0.1 mM Putrescine) | $T_{10}$ Control |

## Appendix – III

Relative economics of different treatments of putrescine under non stress and water stress conditions

See landscape file

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* original not seen

# ABSTRACT

## Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)

Year - 2001-02 & 2002-03

**Investigator: Ms. Sunita Gupta**[*] **MajorAdvisor:Dr.A.K. Purohit**[**]

The study entitled "Antioxidant defense system and lipid peroxidation in relation to drought tolerance in wheat (*Triticum aestivum* L.)" was conducted in laboratory, pot culture and field conditions at SKN College of Agriculture, Jobner during the rabi seasons of 2001-02 and 2002-03 under five experiments viz. (1) To study the effect of water stress on antioxidant enzyme activity and lipid peroxidation in contrasting wheat genotypes (2) To study the changes in antioxidant enzyme activity and lipid peroxidation in relation to putrescence induced drought tolerance in wheat (3) To study physiological attributes of drought tolerance in wheat under laboratory conditions (4) To study physiological attributes of drought tolerance in wheat under pot culture (5) To evaluate the field efficacy of putrescine application.

In the first experiment, polyethylene glycol (PEG) induced water stress significantly increased the superoxide dismutase, peroxidase and catalase activities in 9, 12 and 15 days old seedlings. Genotype C-306 exhibited higher activities of these antioxidants than HD 2329 under water stress conditions. MDA and $H_2O_2$ content significantly increased with increasing PEG concentration and the magnitude was higher in susceptible genotype HD 2329. Among nutrients, nitrogen, phosphorus and calcium content decreased whereas potassium content increased under water stress conditions. Mn and Fe content decreased on account of PEG induced water stress conditions with concomitant increase in Cu content. The C-306 retained higher amount of these nutrients except potassium under water stress conditions.

In the second experiment, putrescine enhanced superoxide dismutase, peroxidase and catalase activities under water stress as well as non stress conditions with higher values in C-306. The MDA and $H_2O_2$ content decreased with putrescine under water stress conditions. The percent reduction was higher in C-306. Putrescine application enhanced the accumulation of nitrogen, phosphorus, potassium, calcium,

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manganese, copper and iron under water stress conditions. The accumulation was better in C-306 as compared to HD 2329.

In the third experiment, PEG induced water stress decreased germination percentage. The genotypic variation was non significant. Root length, shoot length, seedling vigour index, relative water content, membrane stability index, protein and chlorophyll content decreased whereas accumulation of soluble sugars increased significantly under water stress conditions in both the genotypes. A linear reduction in these parameters was recorded with increasing PEG concentration from 10 to 20%. The percent reduction was variable among wheat seedlings of different age i.e. 5, 7, 9, 12 and 15 days old seedlings. The tolerant genotype C-306 showed better performance than drought susceptible genotype HD 2329.

In pot culture, water stress conditions significantly reduced the photosynthesis, transpiration and stomatal conductance in both the genotypes at tillering, anthesis and post anthesis stages. C-306 performed better than HD 2329 under water stress conditions. Internal $CO_2$ concentration increased significantly under water stress conditions. Genotypic variation was also significant. Water use efficiency was also affected on account of water stress.The C-306 retained higher values of water use efficiency under water stress conditions. The relative water content, membrane stability index, chlorophyll and carotenoids decreased whereas sugar and proline content increased under water stress conditions at different growth stages in both the genotypes. Almost similar trend was obtained for these parameters during 2001-02 and 2002-03. Reduction in growth and yield contributing parameters on account of water stress was minimum in C-306 during both the years.

In field experiment, putrescine application enhanced the plant height, leaf area, number of effective tillers, grain weight, grain yield and biological yield under non stress and water stress conditions during 2001-02 as well as 2002-03. exogenous application of 0.1 mM putrescine was found the best in most of the observations. Among mode of applications, two foliar applications of putrescine at the time of anthesis and post anthesis performed the best over seed treatment and one foliar spray of putrescine.

Thus, It is concluded that C-306 appeared drought tolerant at cellular level due to higher antioxidative enzyme activity and lower lipid peroxidation under PEG induced water stress condition. The putrescine might have imparted drought tolerance by enhancing antioxidative enzyme activity and nutrient accumulation. At physiological level C-306 also showed drought tolerant characteristics both at seedling as well as different growth stages such as tillering anthesis and post anthesis stages. The effect of these tolerance mechanisms is clearly perceptible in yield contributing parameters in drought tolerant genotype C-306.The field experiment reflects the significant role of putrescine in increasing productivity of wheat under non stress and water stress conditions. Its effect under field conditions is comparable with cytokinins.

# List of Abbreviations

| S. No. | Name | Symbol |
|---|---|---|
| 1 | Non stress | ns |
| 2 | Water stress | ws |
| 3 | Putrescine dihydrogen chloride | Putrescine |
| 4 | Polyethylene glycol | PEG |
| 5 | Superoxide dismutase | SOD |
| 6 | Peroxidase | POX |
| 7 | Catalase | CAT |
| 8 | Malondialdehyde | MDA |
| 9 | Hydrogen peroxide | $H_2O_2$ |
| 10 | Superoxide radical | $O_2^-$ |
| 11 | Hydroxyl ion | $OH^-$ |
| 12 | Membrane stability index | MSI |
| 13 | Water use efficiency | WUE |
| 14 | Relative water content | RWC |