STUDIES ON THE MECHANISM OF HIGH TEMPERATURE STRESS TOLERANCE IN WHEAT (*Triticum aestivum* L.) GENOTYPES

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STUDIES ON THE MECHANISM OF HIGH TEMPERATURE STRESS TOLERANCE IN WHEAT (*Triticum aestivum* L.) GENOTYPES

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on the mechanism of high temperature stress tolerance in wheat (*Triticum aestivum* L.) genotypes"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** in **Plant Physiology** of the Post Graduate School, Indian Agricultural Research Institute, New Delhi, is a record of *bona-fide* research work conducted by **Mr. Moaed Almeselmani** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation have been duly acknowledged.

Place: New Delhi
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(P.S. DESHMUKH)
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1. INTRODUCTION

Common wheat (*Triticum aestivum* L.) belongs to Poaceae family. It is the most important staple food crop in the world and second important crop in India after rice. Its straw is used as food for large population of cattle in the world. India covers about 32 percent of the total cereals growing area in the world, and is grown in 26.6 million hectares area with the average production of 73 m tonnes and india is the second largest wheat producing nation in the world contributing about $1/9^{th}$ of the global wheat production (Anonymous, 2004-2005). Wheat is now grown extensively in countries with tropical and subtropical climates and the importance of high temperature has been recognized by CIMMYT in Mexico which has a selection programme for high temperature tolerance (Reynolds *et al.*, 1994).

The productivity and yield of wheat is significantly influenced by selection of suitable varieties, soil and environmental conditions as well as the management factors. The environmental stresses like water stress (water logging and drought), temperature (heat and chilling) and salinity are the major problems of wheat growing areas which substantially reduce the yield and quality of wheat (Singh and Purohit, 1995).

Late planting of wheat in India is common due to the intensive cropping system, often delays the sowing of the crop up to the middle of January, particularly in North West India where it is generally sown after harvest of

paddy, sugarcane, pigeonpea and potato. As a result, a portion of maturity period of the crop pushed forward and thus has to face higher temperature of the summer with hot spells often occurring at the time of maturity (Abrol *et al*, 1991). The late sown wheat is more affected by high temperature stress leading to reduced yield and quality (Wardlaw and Veringley, 1994). Morphologically, similar wheat varieties have showed different degree of tolerance to post anthesis high temperature stress. Hence breeding for heat stress tolerance can open new insights significant in Indian agriculture. There are several physiological traits that are associated with heat tolerance, Photoassimilation, chlorophyll retention, chlorophyll a:b ratio, canopy temperature depression, stomatal conductance, membrane stability are some of the examples (Shukla *et al.*, 1997). Photosynthesis, respiration, conversion of sugar into starch in developing grain has been found to be affected by high temperature (Bhullar and Jenner, 1986, Rawson, 1992). It has been reported that high temperature causes membrane damage resulting in electrolyte leakage and this leakage has been shown to be related to temperature and drought tolerance of the wheat genotypes (Deshmukh *et al.,* 1985, 1991 and 1996).

Reynolds *et al.* (1994) showed significant correlation among yield, photosynthetic rate and stomatal conductance and premature loss of chlorophyll associated with heat sensitivity. One mechanism of injury due to heat stress involves the generation and reactions of reactive oxygen species (ROS) (Liu and Huang, 2000). In order to limit oxidative damage under stress condition plants have developed a series of detoxification systems that break down the highly toxic ROS (Larkindale and Huang, 2004). Plants protect cell

and subcellar systems from the cytotoxic effects of the active oxygen radicals using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Sairam *et al.,* 2000). Induction of HSPs in flag leaves of a broad range of winter wheat varieties under field condition has already been reported (Nguyen *et al.,* 1994). Wheat is a sink limited crop and high temperature during grain filling causes the production of shriveled grains due to forced maturity (Savin and Slafer, 1991). Late sown crop gets exposed to mean maximum temperature of about 35 ^{0}C during grain growth and causes yield reduction of 270 kg ha^{-1} per degree rise in temperature (Rane *et al.,* 2000). Temperature adversely alters the growth and development of wheat during the early phase of panicle emergence, grain set and grain development. High temperature reduces the yield drastically due to its detrimental effect on metabolism and duration of phenological phases (Warrington *et al.*, 1977, Saini and Dadhwal, 1986, Acevedo *et al.*, 1990, Jenner, 1991).

Wheat varieties with similar morphological traits have been shown to have differential tolerance to post anthesis high temperature stress. Temperature is the key factor that influences the phenological development and grain yield of wheat crop (Bagga and Rawson, 1977, Rawson and Bagga, 1979, Wardlaw and Wrigley, 1994, Stone and Nicolas, 1994). Wild relatives of crops which have survived under strong natural selection pressure can be particularly useful as a source of genes for specific adaptive traits (Fedak, 1985, Braun *et al.*, 1996). So far genetic variability for heat tolerance was searched only in *T. aestivum* and *T. durum.* Modern wheat varieties are well

adapted to controlled cultural practices, but they are generally not highly tolerant to extreme environmental stresses such as high temperature (Morgunov, 1994).

The physiological approaches of breeding for heat tolerance can provide a tool by identifying the traits that can be easily measured and used as a selection criterion for identifying heat tolerant genotypes. The current investigation has been carried out in order to identify simple techniques which can be used for measurement of heat tolerance mechanism in various wheat genotypes. SO the present study has been planned with the following objectives:

1. To study the physiological and biochemical changes in different wheat genotypes under high temperature stress and involvement of antioxidant defense enzymes in heat stress tolerance.
2. To investigate the extent of damage caused by heat stress on different growth parameters, yield and yield attributes and the correlation between these parameters.
3. Molecular analysis of heat stress tolerance and involvement of HSP 101 in tolerance.

2. REVIEW OF LITERATURE

The optimum temperature for growth and development of each organism differs from each other and depends upon the temperature of its niche, where the organism is evolved. Therefore the upper limits of temperature, an organism can tolerate varies. For example, *Tidestromia oblongifolia*, a C_4 plants, native of Death Vally of California, can photosynthesize even at 50 ^{0}C. Archaebacteria and sulfate reducing bacteria can grow at 110 ^{0}C (Jorgenson *et al.*, 1992). Similarly, Blue green algae can photosynthesize even at 70 ^{0}C. But Eukaryotic organisms have not been found at temperature above 60 ^{0}C (the temperature of hot spring that supports green algae). High temperature stress (5-10 ^{0}C above the normal growing temperature of an organism) is one of the most important environmental stresses that affect the life. Though cell is the fundamental unit of all organisms, the temperature tolerated by some organisms is extremely higher than others. How do the bacteria manage to grow at 110 ^{0}C? It has been suggested that these bacteria have special lipids in the membrane, special conformations of proteins and their DNA is protected by special histone like proteins. When these proteins are added to DNA in vitro, it can withstand temperature 30 ^{0}C higher than the usual (Pool, 1990).

In course of evaluation, the organisms have acquired a whole set of distinct overlapping stress response systems, helping them to survive and

propagate under multitude of unfavorable environmental conditions. Therefore there is a wide range in the temperature tolerance both between species and amongst genotypes within a species. This review will be essentially confined to the latest understanding of the physiological, biochemical and molecular responses of organisms to heat stress and the effect of heat stress on molecular functions, cellular processes, plant growth and development and crop production.

2.1 Physiological and Biochemical Parameters

2.1.1 Chlorophyll Content

Decreases in chlorophyll content due to high temperatures have been observed in rye (Feirabend, 1977) and wheat leaves (Liv and Su, 1985). Bhullar and Jenner (1983) estimated chlorophyll content in the control and warmed ears and reported that chlorophyll content in the pericarp can be taken as an indicator of developmental stage. They further concluded that warming accelerates chlorophyll degradation by about 4^{th} day. Buttery *et al.*, (1981) reported that the variability in photosynthetic rate, nearly 44% was due to variability in chlorophyll content. Xu (1991) reported that photosynthetic rate, thylakoid chlorophyll and protein content of whole, wheat cv. Len, plants were nearly constant at 15/10 ^{0}C but decreased rapidly at 35/30 ^{0}C during maturation. He further observed that chlorophyll content of the flag leaf of wheat cv. Len was also reduced under stress environment (high temperature and moisture), which accelerated the senescence processes and ultimately reduced the grain growth period. Studies of Reynolds *et al.* (1994) showed significant correlation among yield, photosynthetic rate and stomatal conductance and this was probably due to premature loss of chlorophyll

associated with heat sensitivity. Similar association was also given by Condon *et al.*, 1990, Morgan *et al.*, 1993 and Amani *et al.*, 1996. Kislyuk *et al.*, 1997 observed that chlorophyll a : b ratio remained unchanged but the chlorophyll (a + b) contents, calculated on the basis of both, leaf area and dry weight, decreased by 15% after heat treatment.

2.1.2 Photosynthesis

The important response of photosynthesis, among other physiological processes, to changes in temperature, was clearly shown by Bjorkman *et al.* (1974, 1975) working with plants from different ecology. It is well documented that high temperatures inhibit photosynthetic CO_2 fixation and adjustments in the level of unsaturation of thylakoid membranes may affect the capacity of plants to adapt to elevated temperature conditions in order to avoid a reduction in photosynthetic efficiency (Berry and Bjorkman, 1980). While photosynthetic rates were found to be temperature sensitive in other crops, wheat and rice appear to be different. In wheat, no measurable differences were found in photosynthetic rates per unit flag leaf area or on a whole plant basis in the temperature range from 15 to 35 ^{0}C (Bagga and Rawson, 1977) and in rice, there is little temperature effect on leaf carbon dioxide assimilation from 20 to 40 ^{0}C (Egeh *et al.*, 1994). Al-Katib and Paulsen (1990) and Shah, (1992) reported that heat stress decreased mean photosynthetic rates by 32 and 11% in seedlings and mature plants respectively.

The influence of high temperature even in the course of short time results in structural and functional changes of photosynthetic apparatus of higher plants and algae. As for extreme high temperatures, the thylakoid is a distinct sensitive membrane which is inactivated in its function at a lower

temperature compared to other cellular components, like the mitochondria (Thebud and Santarius 1982), and PSII has been recognized as the most sensitive component of the complete photosynthetic system (Berry and Bjorkman 1980) and heat stress damage photosynthetic electron transport, particularly at the site of PSII (Havaux and Tardy, 1996). The reaction center of PSII is considered to be the most sensitive component of the thylakoid membrane to thermal breakdown, and the function of the water-splitting D1 protein within PSII has been implicated as the most readily damaged by high temperature as well as being the primary target for photoinhibition (Aro *et al.*, 1993, Arc *et al.*, 1994). High-temperature induced changes in the membrane composition may therefore play a role in the stability of such proteins leading to positive effects on whole plant growth at elevated temperature. Studies conducted on the ability of plants to acclimate to elevated temperature have mainly focused on components most likely to affect the stability of photosynthetic electron transport, particularly PSII. In this respect, the composition of the chloroplast thylakoids is expected to be important in the thermal tolerance of photosynthetic electron transport (Berry and Bjorkman, 1980).

PSII is the most heat stress susceptible among the photosynthetic reaction centers and enzymes. The heat susceptibility of PSII is studied by measuring the chlorophyll fluorescence in wheat (Sayed *et al.*, 1989 and Mishra and Singhal, 1992) and is related to peroxidation of thylakoid lipids. Chlorophyll (Chl) fluorescence is one of the few physiological parameters that have been shown to correlate with thermo and salinity tolerance. Measurement of PSII activity during leaf heating was used here as a sensitive

indicator of the thermostability that might be conferred by the different membrane compositions (Falk *et al.*, 1996). One of the effects of high temperature on thylakoids, specifically PSII, is the observed sharp rise of the basal fluorescence (F_0, fluorescence emitted by PSII when all PSII complexes are open). The increase in F_0 occurs in plants over a small range of temperatures (40–45 ^{0}C), and has been used as a signal for the irreversible damage of the photosynthetic apparatus. Such increase in the basal fluorescence has been interpreted to be a result of a functional disconnection of the LHC II from the reaction centre and also from a block in the reducing side of PSII (Bukhov *et al.* 1990).

In common bean (*Phaseolus vulgaris L.*) the PSII heat susceptibility studied by measuring O_2 evolution and chlorophyll fluorescence (Chaisompongpan *et al.*, 1990), and it was correlated with heat acclimation potential of different bean genotypes. The quantum yield of PSII depends on its heat stability. The ratio of $\phi H/\phi CO_2$ (ϕH: electron transported via PSII per quantum absorbed, ϕCO_2 : true quantum yield of CO_2 assimilation) was not affected by differences in the biochemical mechanism of CO_2 concentration in bundle sheath cells among five C_4 plants viz., *Flaveria trinervia* (NADP-ME), *Zea mays* (NADP-ME), *Amaranthus cruentus* (NAD-ME), *Panicum maximum* (PEP-CK) and *P. milliaceum* (NAD-ME), and the ratio is lower than a C_3 plant *Elaveria pringlei.* The $\phi H/\phi CO_2$ ratio of C_3 plant increases with raising temperature (Oberhuber *et al.*, 1993). Al-Katib and Paulsen, (1999) compared between Wheat (*Triticum aestivum* L.),

a C_3 species adapted to cool environments: rice (*Oryza sativa* L.), a C_3 species adapted to warm environments; and millet (*Pennesitum glaucum* L.) a C_4 species adapted to hot environments. Photosynthesis was measured in plants grown at 22, 32, or 42 ^{0}C, and light reactions were measured in protoplasts, chloroplasts, and thylakoids isolated from seedlings grown at 27 ^{0}C and treated in vitro at 22, 32, and 42 ^{0}C. Leaf photosynthesis of millet and rice increased from 22 to 32 ^{0}C and then decreased as temperature increases to 42 ^{0}C, whereas in wheat it was highest at 22 ^{0}C and decreased as temperature increased. Photosynthetic rates of protoplasts and chloroplasts from all species decreased after being treated *in vitro* over the same temperature range.

PSII activity declined steadily in protoplasts, chloroplasts and thylakoids of millet and rice from 22 to 42 ^{0}C but decreased abruptly in organelles of wheat from 32 to 42 ^{0}C. The results suggest that differences in photosynthetic responses to high temperature are associated with light reactions and extreme sensitivity of wheat may be attributable to injury to PSII. A higher or lower degree of membrane lipid saturation is beneficial for high temperature tolerance (Klueva *et al.*, 2001). The loss of PSII activity is associated with changes in the distribution of excitation energy between the two photosystems and partly reversible after transfer the leaves and chloroplasts to low temperatures. The rate of oxygen evolution/consumption, *in vivo* electron transport activity, overall photosynthetic capacity were studied after treatment of intact barley seedlings at 40 ^{0}C for 3 hours either in presence of low white light (100 μmol $m^{-2}.s^{-1}$) or in the dark. High temperature impact in the dark resulted in lowering of water splitting capacity,

photosynthetic electron transport rate, suppression of non-photochemical energy dissipation and membrane energization (qE), caused the photoinhibition of PSII at high PFD and indicate impairment of CO_2 assimilation rate in old (11 days old) leaves. The observed data revealed a positive influence of low light in the resistance of the photosynthetic apparatus of barley leaves to high temperature including primary photosynthetic reaction and carbon metabolism. Chen *et al.*, (2004) found that high temperature and strong light stress induced a striking inactivation of PSII particle electron transport and degraded OEC 33 KD polypeptide components.

Photosynthesis decreased in oak (*Quercus pubescens* L.) with increasing leaf temperature and was more than 90% reduced at 45 ^{0}C as compared to 25 ^{0}C. Inhibition of Pn by heat stress did not result from reduced stomatal conductance (g_s), as heat induced reduction of g_s was accompanied by an increase of the intercellular CO_2 concentration (C_i). Chl a fluorescence measurements revealed that between 25 and 45 °C heat dependent alterations of thylakoid associated processes contributed only marginally, if at all, to the inhibition of Pn by heat stress, with PSII being remarkably well protected against thermal inactivation. Inhibition of Pn at high leaf temperature could be markedly reduced by artificially increasing C_i. A high C_i also stimulated photosynthetic electron transport and resulted in reduced non photochemical fluorescence quenching (Haldimann and Feller, 2004). Gupta and Gupta (2005) reported that the high temperature adversely affects the photosynthetic functioning and the thylakoid membrane particularly the PSII complexes located on the membrane, which are most heat sensitive part of

the PSII mechanism. In addition, Rubisco and other enzymes of carbon metabolism are also adversely influenced by high temperature.

2.1.3 Rubisco

Weis (1981b) reported that light-dependent activation of Rubisco in spinach (*Spinacia oleracea* L.) chloroplasts was inhibited by moderately elevated temperatures and that inhibition was closely correlated with reversible inhibition of CO_2 fixation. A similar effect of temperature on Rubisco activation and CO_2 fixation was reported for wheat (*Triticum aestivum* L.) leaves (Kobza and Edwards, 1987). The temperature induced inhibition of carbon assimilation cannot be explained solely by increased photorespiration via the enhanced specificity of Rubisco for oxygen at higher temperatures (Jordan and Ogren, 1984). The synthesis of RuBPcase/oxygenase, the most abundant protein on the earth is susceptible to heat stress. In green suspension cultures of soybean, when the temperature is raised 38-40 ^{0}C the amount of RuBP decreased 20-30% (Nover *et al.*, 1989). The small subunit synthesis is more heat susceptible than larger subunit in field grown soybean. There exist genetic differences even within a species for heat tolerance in Rubisco SSU synthesis. At 34 ^{0}C, the heat tolerant wheat variety mustang was able to maintain higher level of SSU synthesis than the heat susceptible variety sturdy (Krishnan *et al.*, 1989). The sensitivity of SSU mRNA to heat shock suggests that decreased synthesis of chloroplast proteins produced in the cytoplasm may be an important causal factor of heat damage to plants (Nover *et al.*, 1989). However, after one-hour heat acclimation at 37 ^{0}C, wheat varieties did not show any difference in SSU synthesis. This may be results of HSP induction at 37 ^{0}C and protection of transcriptional and translational

machinery by HSPs (Krishnan *et al.*, 1989). In soybean leaves the degree of Rubisco activation did not show a clear correlation with either CO_2 concentration or with air temperature, whereas the concentration of RuBP in the upper canopy leaves decreased with increasing air temperature from 26 to 36 ^{0}C (Campbell *et al.*, 1990). The reduction in the RuBP concentration at high temperature may be due to the increased rate of oxygenation reaction catalyzed by the Rubisco at high temperature (Ogren, 1984). Sharkova (1994) observed that Rubisco activity was not significantly altered even after the heat treatment which induced severe damage to photosynthesis and photosynthetic electron transport. Increase in temperature from 20 ^{0}C to 25 ^{0}C increased photosynthesis with a Q_{10} value of 1.90. As the temperature increased from 25 ^{0}C to 30 ^{0}C the Q_{10} decreased possibly following a restriction in the regeneration of the acceptor for CO_2 and as temperature increased from 30 to 35 ^{0}C Q_{10} was further decreased, as the result of the inability of the thylakoid to maintain adequate supply of NADH (Pastenes and Horton, 1995).

Feller *et al.*, (1998) showed that light activation of ribulose-1, 5-bisphosphate Rubisco is inhibited by moderately elevated temperature through an effect on Rubisco activase. When cotton (*Gossypium hirsutum* L.) or wheat (*Triticum aestivum* L.) leaf tissue was exposed to increasing temperatures in the light, activation of Rubisco was inhibited above 35 and 30 ^{0}C, respectively, and the relative inhibition was greater for wheat than for cotton. The temperature induced inhibition of Rubisco activation was fully reversible at temperatures below 40 ^{0}C. In contrast to activation state, total Rubisco activity was not affected by temperatures as high as 45 ^{0}C.

Nonphotochemical fluorescence quenching increased at temperatures that inhibited Rubisco activation, consistent with inhibition of Calvin cycle activity. Initial and maximal chlorophyll fluorescence was not significantly altered until temperatures exceeded 40 ^{0}C. Thus, electron transport, as measured by Chl fluorescence, appeared to be more stable to moderately elevated temperatures than Rubisco activation.

Moderately elevated temperatures have a direct effect on the distribution and form of activase, the biochemical component controlling Rubisco activation. A sensitivity of activase to inactivation at moderately elevated temperatures is consistent with the poor thermal stability of the isolated enzyme (Robinson and Portis, 1989, Holbrook *et al.*, 1991, Crafts-Brandner *et al.*, 1997; Eckhardt and Portis, 1997).

For both cotton and wheat, a 5 min exposure of leaf tissue to temperatures of 30 to 35 ^{0}C had a rapid and readily reversible effect on light activation of Rubisco Weis (1981a). Weis (1981a, 1981b) and Kobza and Edwards (1987) observed a close relationship between loss of Rubisco activation and inhibition of CO_2 fixation in response to increasing temperature. Salvucci and Ogren, (1996) suggested that increases in qN that were observed upon exposure of cotton and wheat to elevated temperatures were attributable to the consequent effects of activase inhibition on Rubisco activation and CO_2 fixation. Weis (1981a) reported an increase in thylakoid energization (a major component of qN) in response to loss of Rubisco activation at elevated temperature. Similarly, Salvucci *et al.* (1987) showed that qN and light scattering were both increased under ambient conditions in a

mutant of Arabidopsis that is unable to activate Rubisco because of a lack of activase.

Crafts-Brandner *et al.* (1997) reported that high temperature physically perturbs activase, leading to an inhibition of enzyme activity and the consequent effect on light activation of Rubisco. The effect of high temperature stress on the expression of ribulose-1, 5-bisphosphate Rubisco activase was examined in wheat (*Triticum aestivum* L.) leaves, which normally possess 46 and 42 kDa activase forms. Heat stress at 38 ^{0}C significantly reduced total activase mRNA levels compared to controls, and recovery of activase transcription was only marginal 24 h after alleviating heat stress. In contrast to transcript abundance, immunoblot analysis indicated that heat stress increased the accumulation of the 42 kDa activase and induced a putative 41 kDa form. Heat stress did not affect the amounts of the 46 and 42 kDa activase forms (present as 51 and 45 kDa preproteins) recovered after their immunoprecipitation from *in vitro* translation products. *De novo* protein synthesis *in vivo* in the presence of [35 S] Met/Cys showed an increase in the amount of newly synthesized 42 kDa subunit after 4 h of heat stress, and synthesis of the putative 41 kDa activase was apparent. In contrast to activase, heat stress led to a rapid and large reduction in the *de novo* synthesis of the large and small subunits of Rubisco. Long term (48 h) heat stress further increased the amounts of *de novo* synthesized 42 and 41 kDa activase forms. After 24 h of recovery from heat stress, *de novo* synthesis of the 42 kDa activase returned to control levels, while a small amount of 41 kDa protein was still expressed. Southern analysis suggested the presence of a single activase gene. This indicated that heat stress alters activase

expression, most likely post transcriptionally, and suggest that the heat induced expression of the 42 and 41 kDa subunits of wheat leaf Rubisco activase may be related to the maintenance and acclimation of photosynthetic CO_2 fixation during high temperature stress in wheat (Law and Crafts, 2001).

Rubisco activation in tobacco protoplasts decreased at temperatures higher than 30 ^{0}C .The same point in the temperature response where the activities of isolated Rubisco and activase began to deviate. The first indications of thermal denaturation of activase both *in vitro* and *in vivo* occurred at temperatures near the optimum for ATP hydrolysis (42 ^{0}C - 44 ^{0}C) and denaturation was extensive at temperatures above the optimum. Because activase physically interacts with Rubisco, minor changes in its structural integrity or oligomeric state could affect its ability to interact productively with Rubisco. A disruption of activase-Rubisco interactions would explain the inability of activase to maintain Rubisco in an active state at temperatures between 30 ^{0}C and 44 ^{0}C, i.e. elevated temperatures that are at or below the temperature optimum for ATP hydrolysis. In an alternate manner, activase activity may simply be inadequate to offset the faster rate of Rubisco deactivation at these temperatures (Crafts-Brandner and Salvucci, 2000).

At temperatures higher than the optimum for ATP hydrolysis, thermal denaturation of activase was extensive. As a consequence, the marked decrease in Rubisco activation at temperatures greater than 44 ^{0}C is almost certainly caused by loss of activase activity per second and disruption of activase Rubisco interactions. Compared with other chloroplast proteins, activase was extraordinarily sensitive to thermal denaturation. In pea, which has two forms of activase, the shorter form was considerably more sensitive to

thermal denaturation and aggregation than the longer form. Similar results were reported for the two forms of activase in spinach leaf discs (Rokka *et al.*, 2001).

In Arabidopsis plants subjected to abrupt heat stress, both forms of activase exhibited similar patterns of thermal aggregation. Thus, species variability may exist in the relative sensitivities of the two-activase polypeptides to thermal denaturation. When heat stress is imposed rapidly, activase rapidly loses structural integrity, probably overwhelming the constitutive chaperonin system. However, if heat stress is imposed slowly photosynthesis can acclimate, requiring higher temperatures for inhibition of CO_2 fixation (Berry and Bjorkman, 1980, Weis and Berry, 1988) and for deactivating Rubisco (Law and Crafts-Brandner, 1999).

The mechanistic basis for photosynthetic acclimation is unknown, but could involve stabilization of activase structure by chaperonins to prevent activase from participating in unproductive associations with other activase molecules or with Rubisco or other chloroplast proteins. In an alternate manner, *de novo* synthesis of more thermally stable forms of activase (Sanchez de Jimenez *et al.*, 1995, Law and Crafts-Brandner, 2001), modifications that improve the thermal stability of existing forms of activase or changes in expression that increase the ratio of the longer, more thermally stable form of activase (Crafts-Brandner *et al.*, 1997) could also provide mechanisms for photosynthetic acclimation to high temperature. A more permanent way of increasing the thermal stability of photosynthesis may be to transform plants with additional copies of activase or with an activase that is engineered to be more thermally stable. Modification of activase structure to

improve its thermal stability will require changes that do not compromise the ability of activase to interact with Rubisco.

Salvucci *et al.*, (2004) reported that the formation of high-molecular-weight aggregates of activase after exposure of intact leaf tissue to high temperature provided direct evidence that the physical structure of the enzyme was perturbed by the temperature treatment. Activase from wheat was much more susceptible to structural damage compared with cotton, similar to the results for Rubisco activation, and Chl fluorescence. The temperature required perturbing the distribution and form of activase was much higher than the temperature that caused reversible inhibition of Rubisco activation, occurring at temperatures that caused irreversible damage.

2.1.4 Respiration

Respiration is a process, which conserves energy for cellular function, growth and differentiation. In wheat, dark respiration rate at 20 ^{0}C is about 1/3 of net photosynthetic rate at each stage of growth and it increases upon temperature upshift. For most plant species the Q_{10} for respiration between 5-28 ^{0}C is 2 to 2.5. Upto 35 ^{0}C the rate of respiration increases and Q_{10} begins to decrease. Therefore, heat stress can reduce the net carbon fixed by doubling the dark respiration, even within the physiological range. Dark respiration rate of wheat increases from 0.3 mg CO_2 $dm^{-2}h^{-1}$ to 2.5 mg CO_2 $dm^{-2}h^{1}$ upon temperature upshift from 15 ^{0}C to 35 ^{0}C and thus contributes to considerable yield loss (Evans *et al.*, 1973). The electron transport and oxidative phosphorylation was less affected at 32 ^{0}C in *Phaseolus acutifolius* A. Gary than in *P. vulgaris* L. This lower mitochondrial efficiency of *P. vulgaris* resulted in reduced plant growth at 32 ^{0}C. Therefore, thermostability of

mitochondrial functions may determine the high temperature tolerance in plants (Lin and Markhart, 1990).

2.1.5 Membrane Thermostability

The two major sites potentially affected by high temperatures are enzymes and membrane. Membrane disruption may alter water, ion and organic solute movement, photosynthesis and respiration (Christiansen, 1978). Martineau *et al.*, (1979) observed that when leaf tissue was injured by high temperature, membrane damage was increased and electrolytes diffused out of cells. Blum and Ebercon (1981) estimated drought and heat tolerance of sorghum by measuring the electrical conductivity of aqueous media containing the leaf disc that were previously treated with polyethylene glycol and concluded that relationship between the degree of leakage and tolerance to drought and heat stress did not exist. Other workers have also used conductometers measurements of solute leakage from cells to estimate heat damage to plasma membranes (Sullivan and Ross, 1979, Singh *et al.*, 1991). Blum and Ebercon (1981) observed genetic variation in membrane thermostability using conductometric measurements in various field crops including spring wheat. The heat susceptibility of plasma membrane has been shown by ion leakage studies in many crop plants. The increased leakage of solutes is an indication of damage to membrane (Chaisompongopan *et al.*, 1990). Heat injury to the plasmalemma may be measured by ion leakage (Chaisompongpan *et al.*, 1990, Hall, 1993). However, stable cell membrane that remains functional during stress appears to control adaptation to high temperature and found related to heat and drought tolerance (Sullivan and Ross, 1979, Raison *et al.*, 1980). Blum (1988) reported that membrane is one

of the sites of primary physiological injury by heat. A proportional change in ion leakage with increasing temperature in tissues of wheat was reported by (Navari- Izzo *et al.*, 1993). Deshmukh *et al.*, (1985, 1991, 1996) have also suggested the use of ion leakage as a measurements index for screening genotypes against heat and drought stress in wheat. They observed that with increase in temperature there was a proportional increase in ion leakage. Nagarajan and Rane (1997) also emphasized the use of membrane thermostability as one of the simple parameter for screening wheat genotypes against high temperature stress. The ability of plants to acclimate to higher temperatures was conducted on plants adapted to high temperature growth (Berry and Bjorkman, 1980 and Pearcy, 1977).

The desert shrub *Atriplex lentiformis*, changes its membrane fatty acid composition by decreasing the level of unsaturated fatty acids such as hexadecatrienoic acid (16:3) and increasing the level of saturated lipids at higher growth temperatures (Pearcy, 1978). Membrane fatty acids of plants from temperate environments show similar trends in response to temperature, an observation that suggests that alterations in membrane lipids generally contribute to the ability of plants to acclimate to different temperatures (Harwood *et al.*, 1994, Williams *et al.*, 1988). The plasmalemma and membranes of cell organelles play a vital role in the functioning of cells. Any adverse effect of temperature stress on the membranes leads to disruption of cellular activity or death. Injury to membranes from a sudden heat stress event may result from either denaturation of the membrane proteins or from melting of membrane lipids which leads to membrane rupture and loss of cellular contents (Ahrens and Ingram, 1988).

The susceptibility of biomembranes to sudden heat stress may be due to:

1) Denaturation of membrane proteins

2) Melting of lipids in membrane which leads to breakage of membrane and loss of cellular contents. Long term acquisition of heat tolerance in several plants is accompanied by a decrease in fatty acid unsaturation resulting in a decrease in the lipid fluidity and an increase in the phase transition temperature. This leads to the stability of membrane during heat stress. Lipid unsaturation is a component of heat tolerance in fad mutants of Arabidopsis in which a pronounced decrease in trienoic fatty acids of chloroplast membrane results in heat tolerance while large increase in the lipid saturation had only minor effects on the rate of photosynthetic electron transport (Somerville and Browse, 1991).

The fad B mutant of Arabidopsis deficient in the chloroplast fatty acid "w9 desaturase" accumulates high amounts of 16:0 fatty acids, and shows an overall reduction in the level of unsaturation of chloroplast lipids. In short term growth tests, fad B mutant had a substantially higher growth rate than the wild type plants at the highest temperature tested, suggesting that chloroplast membrane thermal stability is a component of thermal tolerance in the whole plant (Kunst *et al.*, 1989). Heritability of a trait expresses the reliability of the phenotypic value as a guide to the breeding value and the trait utility within the selection process (Saadalla *et al.*, 1990).

The chloroplast membrane of higher plants contains an unusually high concentration of trienoic fatty acids. Plants grown in colder temperatures have a higher content of trienoic fatty acids. Transgenic tobacco plants in which the gene encoding chloroplast omega-3 fatty acid desaturase, which synthesizes

trienoic fatty acids, was silenced contained a lower level of trienoic fatty acids than wild-type plants and were better able to acclimate to higher temperatures (Murakami *et al.*, 2000). Ibrahim and Quick (2001) suggested that heat tolerance based on membrane thermal stability can be improved using the existing genetic variability available within germplasm. Talwar (2002) and Singh *et al.*, (2004) observed that membrane stability is one of the important parameters to evaluate genetic variability for heat stress.

2.1.6 Water Status

Heat stress injury involves water deficit and cell turgor loss (Ahmad *et al.*, 1989). Maintenance of favorable water status is essential for plant tolerance to heat stress (Graves *et al.*, 1991, Lehman and Engelke, 1993 and Jiang and Huang, 2000). A heat tolerant cultivar of cotton was able to survive heat stress by accumulating solutes to maintain cell turgor (Ashraf *et al.*, 1994). Drought preconditioning-enhanced heat tolerance may be related to the maintenance of plant water relations by reducing water loss and or increasing water uptake capacity. Osmotic adjustment is well known to be an important physiological mechanism of water retention and cell turgor maintenance (Turner and Jones, 1980 and Morgan, 1984). Relative water content (RWC) has been considered as a better indicator of water status as it reflects the balance between water supplied to leaves and transpiration rate through its relation to cell volume (Sinclair and Ladlow, 1985). Due to high temperature induced higher transpiration, situation similar to water stress is created and RWC becomes important in heat stress also. Spomer (1985), while discussing the different conventional parameters for measuring plant water status, advanced the opinion that water potential is probably the best

single measure of the water status in plants. Water potential, ψ, describes water status in terms of its availability for plant processes.

NMR relaxation times have been used widely to describe the water status as well as heat and freezing injury in plant tissues (Rajashekar and Burke, 1986 and Abass and Rajashekar, 1991). Nuclear magnetic resonance (NMR) offers a non-invasive method for characterization water status in biological tissues (Lewa and Lewa, 1990). Membrane disorganization leads to changes in molecular mobility and other biophysical properties of tissue water (Maheswari *et al.*, 1999). The ^1H NMR transverse relaxation time T_2 of the tissue water has been shown to be related to the water content of the plant tissue, properties of water in different parts of the tissue and interactions with macromolecules (Hills and Duce, 1990, Ratciffe, 1994). It has been shown that proton spin-lattice relaxation time T_1 of leaves of barley (Colire *et al.*, 1988) and wheat (Nagarajan *et al.*, 1993) is a good indicator of plant water status than conventional water relation parameters (Nagarajan *et al.*, 1996).

Sairam *et al*, (2000) reviewed that extent of oxidative injury and activity of antioxidant enzymes in relation to heat stress induced by manipulation dates of sowing and increase in temperature by late sowing significantly decreased leaf relative water content, ascorbic acid content and increased H_2O_2 content at 8 and 23 days after anthesis. Paulsen and Machado (2001) observed that high temperature interacts with drought to affect water relations and the effect was greater in heat sensitive wheat (*Triticum aestivum*) than in sorghum.

2.1.7 Assimilation Rate

2.1.7.1 Assimilate Supply

Since the number of endosperm cells is decided by the assimilate supply (Brocklehurst *et al.*, 1978), the availability of assimilate is a major factor that determines the final grain weight. Heat stress affects the assimilate availability mainly through its effects on the photosynthesis, rate of respiration and translocation of photosynthates to the grain (Acevedo *et al.*, 1990). There are two sources of assimilate supply for grain growth: photosynthesis of flag leaf and ear after anthesis, and translocation of materials assimilated before anthesais (Gallagher *et al.*, 1976). As already discussed, due to the high temperature the rates of flag leaf photosynthesis decreased (Al-Katib and Paulsen, 1984) and respiration rate increased approximately 7 times upon temperature upshift from 15 to 25 ^{0}C. In wheat ear photosynthesis may contribute to the final grain dry weight upto 50% (Evans *et al.*, 1973) and presence of awns doubles the rate of net photosynthesis by ear. Presence of awns is an important component of thermotolerance, because of its photosynthetic stability at high temperatures (Blum, 1986).

Under irrigated warm condition, the reserves of carbohydrates are not mobilized to grain (Jenner, 1991) as well as harvest index of most wheat cultivars is reduced at high temperature (Ruwali and Prasad, 1991), which may be due to :

1) Inhibitory effect of heat on translocation of assimilates.

2) Inactivation of starch synthase at high temperatures (Rijven, 1986, Mohabir and John, 1988). Hence the sugars accumulate which reduce the phloem unloading and thus reduce the sink demand resulting in decreased

partitioning to the grain at high temperature. The sink limitation in turn may also reduce the source strength (Evans *et al.*, 1973, Sinha and Khanna, 1975) and thus contribute to lower yield.

2.1.7.2 Starch Synthesis

The rate and duration of starch deposition in wheat grains determines the final grain weight and is determined by the capacity of plant to provide photoassimilates and the capacity of grains to synthesize starch (Jenner, 1991). Among the environmental stresses high temperature is most deleterious, which reduces the rate as well as the duration of starch synthesis (Gallagher *et al.*, 1976). Among the enzymes involved in starch synthesis, following two are most susceptible to high temperature:

1) Starch synthase of wheat (Rijven, 1986, Bhullar and Jenner, 1986), potato (Mohabir and John, 1988).

2) Sucrose synthase of wheat (Jenner, 1991) and barley (McLead and Duffus, 1988). Sucrose synthase converts the sucrose to UDP-G and fructose. UDPG is then converted to ADPG, which is then converted to starch by starch synthase. Both enzymes from wheat and potato (cool season crops) are highly susceptible to high temperatures (35 ^{0}C) whereas the starch synthesis in rice is stable even at 39/34 ^{0}C (Tashiro and Wardlaw, 1991). Between wheat cultivars, there is variation in tolerance of starch deposition to elevated temperatures (Wardlaw *et al.*, 1989). Therefore, there is opportunity to manipulate heat tolerance by conventional breeding or by transferring genes coding for more heat tolerance enzymes, e.g. rice by genetic engineering (Jenner, 1991).

Wallwork *et al.* (1998) studied the effect of high temperature during grain filling on starch synthesis in the developing barley grain. Plants of malting barley were exposed to 5 days of high temperature (up to 35 ^{0}C) during mid grain filling under controlled environmental conditions. Grains from heat treated plants accumulated 30 per cent less starch than grains from control plants (21/16 ^{0}C, 14 hrs day). Their findings suggested 11-75% reduction in activity of the enzymes under investigation followed by high temperature exposure. In addition, ADP glucose pyrophosphorylase, branching enzyme and granule bound starch synthase showed increased activity during exposure to moderate temperatures (28-32 ^{0}C), but reduced activity at high temperatures, while soluble starch synthase showed an immediate loss of activity, even at moderate temperatures. Sucrose synthase and UDP glucose pyrophosphorylase showed the greatest reduction in catalytic activity after plants was returned to cooler conditions.

Wilhelm *et al.* (1999) studied the effect of extended period of high temperature during grain filling on kernel growth, composition, and starch metabolism of seven maize genotypes. Reduction in kernel growth rate resulted in an average mature kernel dry weight loss of 7%. Proportionally similar reductions occurred for starch, protein and oil contents of the kernel. A survey of 11 enzymes of sugar and starch metabolism extracted from developing endosperm revealed that ADP glucose pyrophosphorylase, glucokinase, sucrose synthase and soluble starch synthase were most sensitive to the high temperature treatment. Prakash *et al.* (2003) observed that high temperature (+5-7 ^{0}C) during grain development phase decreased grain growth in wheat (*Triticum aestivum*) cultivars. Associated with a

decrease in grain growth under elevated temperature (ET), there was a decrease in grain starch content and soluble starch synthase (SSS) activity. SSS activity decreased by ET, 20 DAA, a stage beyond which both grain growth and starch accumulation in grains began to decrease at ET compared to control. Relatively tolerant cultivars showed less decrease compared to susceptible types in grain growth, starch accumulation and SSS activity. Out of soluble and granule bound starch synthesis, activity of SSS was found to be more sensitive to high temperature. Prasad *et al.* (2004) also observed that when excised developing grains were pre exposed to gradual rise in temperature from 15 to 45 ^{0}C, the decrease in SSS activity was less compared to direct exposure to 45 ^{0}C.

Zehedi *et al.* (2003) reported that in two wheat cultivars differing in tolerance to high temperature, final grain weight was reduced by 33% in the least sensitive (cv. Kavko) and by 40% in the most sensitive (cv. Lyallpur) cultivar as post anthesis temperature was raised from 20/15 ^{0}C (day/night) to 30/25 ^{0}C. The difference in the response of the two cultivars was mainly due to changes in the rate of grain filling at high temperature. The response of the rate of grain filling at high temperature and the differential effects on the two cultivars did not seem to be explained by an effect of temperature on the supply of assimilate (sucrose) or on the availability of the substrate (ADPG) for starch synthesis in the grains. *In vitro* but not *in vivo*, the differential responses of the efficiency (V_{max}/K_m) of SSS in the two cultivars to an increase in temperature were associated with differences in the temperature sensitivity of grain filling. *In vivo*, the most remarkable difference between the

two varieties was in the absolute values of the efficiency of SSS, with the most tolerant cultivar having the highest efficiency.

2.1.7.3 Grain Growth Rate and Duration

To get 30 days of grain filling duration, anthesis must occur by 15 December to early January in central India and in south India. Temperature increase from 15/10 ^{0}C to 21/16 ^{0}C reduced the duration of grain filling from 60 to 36 days. Growth rate per grain depends on floret position within the ear and growth rate varies between cultivars and increases with rise in temperature. (Sofield *et al.*, 1977). Temperature has a pronounced effect on duration of grain filling. At low temperature, grain development continues for a longer period. This results in higher final grain weight (Warrington *et al.*, 1977). The heat degree days required for wheat grain to attain 95% of the maximum harvest weight from date of anthesis is around 400 heat degree days (HDD) for HD 4152, Kalyansona and sonalika. Therefore, high temperatures during this phase will provide 400 HDD in less days and thus the duration of grain development is reduced (Saini and Dadhwal, 1986). The response of grain filling to high temperature appears to have a direct effect on the grain itself as the response is largely independent of the source sink balance (Wardlaw *et al.*, 1989). Rate and duration of grain filling determine final grain weight (Duguid and Brule, 1994). Kernel dry weight at maturity is positively correlated with the rate of kernel filling (Wardlaw and Moncur, 1995).

2.1.8 Antioxidant Enzymes Activity

Environmental stresses induce production of active oxygen species such as superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and

singlet oxygen ($^1O_2$). They are highly reactive and can damage many important cellular components such as lipids, proteins, and nucleic acids in living cells (Smirnoff, 1993, Foyer *et al.*, 1994). Plants have developed enzymatic and nonenzymatic scavenging systems to quench active oxygen species. When plants are subjected to stresses such as high temperatures, the scavenging system may lose its function and the balance between producing and quenching active oxygen species can be disturbed, resulting in oxidative damage (Price *et al.*, 1989, Bowler *et al.*, 1992, Zhang and Kirkham, 1994).

High temperature induced oxidative stress in various higher and lower plants (Upadhyaya *et al.*, 1990, Jagtap and Bhargava, 1995, Davidson *et al.*, 1996) and heat injury in cool season grasses has been associated with oxidative damage (Jiang and Huang, 2001 and Liu and Huang, 2000). Physiological injury due to heat stress has been associated with increases in oxidative damage in perennial grasses (Liu and Huang, 2000) and other plant species (Larkindale and knight, 2002). Heat stress has been shown to cause oxidative stress due to over production of active oxygen species; such reactive molecules cause cellular damage, particularly to cell membrane, and Oxidative protection considered as an important component in determining the survival of plant during heat stress (Larkindale and Huang, 2004).

Tolerance to high temperature stress in crop plants have been associated with an increase in antioxidant enzymes activity (Rui *et al.*, 1990, Gupta *et al.*, 1993, Badiani *et al.*, 1994 and Zhau *et al.*, 1995). Several enzymatic and non enzymatic antioxidant defense systems maintain AOS concentrations under tight control to protect cells from damage (Noctor and

Foyer, 1998). Sairam *et al.*, (2000) reported that plants protect cell and subcellular systems from the cytotoxic effects of these active oxygen radicals using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and metabolites like glutathione, ascorbic acid, tocopherol and carotenoids. The activity of enzymes associated with the antioxidant defense system, especially ascorbate peroxidase has been shown to increase rapidly under heat stress in mustard (Dat *et al.*, 1998).

2.1.9 Heat Shock Protein

Under heat stress conditions, the normal cellular protein synthesis will decrease with concomitant increase in HSP synthesis in all organisms. However different organs in an organism as well as different species have different responses to heat stress. Heat shock causes a drastic decrease of normal cellular proteins synthesis in soybean and maize leaves but causes very subtle change in maize root tips. However, in wheat and rice grains, protein synthesis is largely unaffected although an increase in grain protein may result at high temperatures (Bhullar and Jenner, 1985).

One of the most prevalent environmental challenges encountered by plants is the exposure to a broad range of temperatures. Plants use a variety of anatomical, metabolic and cellular strategies to deal with changing environmental temperatures. Acclimation to elevated temperatures is mediated at the cellular level in part by the induction of general stress responses, which include the increased expression and activity of heat shock proteins (Gallie *et al.*, 1998). Heat stress (5-10 ^{0}C above the normal growth temperature of organisms) induces expression of specific gene families called

heat shock genes (hsps), which lead to synthesis of heat shock proteins (HSPs), in every organism in which it has been sought, from unicellular prokaryote to highly evolved homo sapiens. The only exception to this is the germinating pollen of higher plant, which does not synthesize HSPs during heat stress (Farova *et al.*, 1989). All the species examined so far are able to acquire tolerance to lethal temperatures, if the cell previously exposed to non lethal hsp inducing heat shock (Schlesinger, 1990, Vierling, 1991). This acquired thermotolerance occurs very rapidly, with concomitant synthesis of HSPs, which require a few minutes of heat shock induction. HSPs express from within 1-3 hours of heat shock and decay 12-48 h after (Nover *et al.*, 1989). The HSPs can be classified based on their molecular weight into following families:

1) HSP 110 (100 to 110 KDa)
2) HSP 90 (85 to 95 KDa)
3) HSP 70 (70 to 80 KDa)
4) HSP 60 (50 to 68 KDa)
5) HSP 20 (15 to 28 KDa)
6) Ubiquitin (8.5 KDa)

The high molecular weight (HMW) HSPs ranging from 68-110 KDa are ubiquities among all organisms, where as the low molecular weight (LMW) HSPs (HSP 20 family) predominate in higher plants (Vierling, 1991).

Some members of HSP 90, HSP 70 and HSP 60 are expressed not only during normal cell growth and development but also induced by other stresses such as toxic metals, ethanol etc. (Howarth, 1990). This argues for their roles in normal functioning of cell during optimal and stressed

environment. Constitutive HSPs (HSCs) have weak ATPase activity, which may be essential for its role in protein transport, protein folding and assembly (Pelham, 1986).

ATP dependent "unflodase" activity of HSPs is essential and regulates its function as molecular chaperone (Miernyk *et al.*, 1992). The DnaK mutant of *E.coli* shows impaired DNA synthesis and cell division. HSP 70s also have calmodulin binding site (Schlesinger, 1990). Therefore, during the growth of an organism in its optimal environment, the HSPs are playing an essential role in DNA replication (Craig and Cross, 1991), transcription, translation, transport, folding, unfolding and assembly of protein and regulation of activities of proteins. Besides this, HSP 70 regulates the HSPs synthesis upon heat stress (Craig and Cross, 1991 and Morimoto, 1993).

2.1.9.1 Role of HSPs in Thermotolerance

The phenotypic characterization of HSP mutants has provided strong evidence that the HSPs are an essential component of thermotolerance. The hin mutant of *E. coli* (Yamamori and Yura, 1982), HSP 104 mutant of yeast (Sanchez and Lindquist, 1990) and SSA1 and SSA2 mutant of yeast were unable to survive in lethal temperatures, even after giving a prior hsp inducing non-lethal heat shock. But the wild types could survive.

The cells could not acquire thermotolerance, where HSP synthesis is either blocked at transcriptional (Johnston and Kucey, 1988) and/or translational level (Yamamori and Yura, 1982) or inactivated by antibodies (Riabowl *et al.*, 1988). HSPs induced by other stresses can also provide thermotolerance (Howarth, 1990), which provides further evidence for the involvement of HSPs in thermotolerance.

HSPs are essential component of acquired thermotolerance of germinating seeds (Abernethy *et al.*, 1989) and seedling growth of wheat (Blumenthal *et al.*, 1990, Krishnan *et al.*, 1989) soybean (Lin *et al.*, 1984) sorghum and pearlmillet (Ougham and Stoddart, 1986, Howarth, 1990). However, the exact mechanism by which HSPs provide thermoprotection is still obscure. HSPs may give thermotolerance to an organism by protection of nucleus, ribosome synthesis (Schlesinger, 1990) protein synthesis (Krishnan *et al.*, 1989), soluble proteins (Hsich *et al.*, 1992), thylakoid membranes of chloroplast (Schuster *et al.*, 1988) and electron transport chain of mitochondria (Chon *et al.*, 1989) during heat stress. The role of HSPs in thermotolerance is not incontrovertible (Apuya and Zimmerman, 1992, Ramsay, 1988, Susek and Lindquist, 1989). However, if these studies are closely examined, only a group of HSPs appear to be involved in thermotolerance. Since in plants and other organisms, there are 6 major HSP families, each of which consists of upto 15 hsps, all of them may not be required for thermotolerance at all stages of growth.

2.1.10 Senescence

The final grain yield is associated with the leaf area duration. The leaf area duration depends on the onset of senescence. The sink demand (Guitman *et al.*, 1991) as well as the high temperature stress induces senescence in wheat (Kuroyanagi and Paulsen, 1985). The normal increase in leaf protease activity and changes in rates of other processes during senescence of wheat are greatly slowed by low temperature and speeded by high temperature (Al-Katib and Paulsen, 1984). The mode of temperature-induced senescence may be through heat stress induced increase in ABA

and ethylene (Nover *et al.*, 1989). Comparison of wheat senescence with and without influence suggests that main cause for the accelerated senescence is high temperature (Kuroyanagi and Paulsen, 1985). **The heat-induced damaging processes increased considerably with leaf senescence whereas the young seedlings were the most adaptive and flexible to the environment (Kalituho** *et al.*, .2003).

2.2 Growth Parameters

2.2.1 Leaf Area Index

Leaf area index was positively correlated with chlorophyll content and yield and negatively correlated with plant height (Kler and Bains, 1989). Leaf area index showed significant positive association with leaf area ratio (LAR) and leaf area duration (LAD) but negative association with ear number per plant (Sharma *et al.*, 1991). Das *et al.*, (1993) found peak value of LAI, 73-85 days after sowing irrespective of sowing rate and nitrogen rate. Ostapenko (1993) concluded that final yield was highly correlated with leaf area index and photosynthetic potential. Sharma *et al.*, (1994) observed that LAI had high degree of association with grain yield. Frederick and Camberato (1995) found that rate of decline in LAI during grain fill were more rapid in 1993 compared to 1994, when air temperature was relatively warmer. Heat stress reduces the duration of vegetative growth (Saini, 1988) and therefore reduces leaf area (Warrington *et al.*, 1977) and leaf number (Acevedo *et al.*, 1990).

2.2.2 Crop Growth Rate

Sharma *et al.* (1991) have observed significant positive association of crop growth rate (CGR) with relative growth rate (RGR) and dry weight per ear and negative association of RGR with tillers number per plants. Karimi

and Siddique, (1991) considered that higher grain yields of modern cultivars of wheat was achieved with higher RGR during the negative phase and greater CGR from ear emergence to harvest. Delayed sowing decreased crop growth rate and net assimilation rate during the late grain filling period in wheat cultivars and decreased biomass (Takahashi and Nakaseko, 1992). Simane *et al.* (1993) observed that drought resistant wheat cultivars had high RGR in favorable period of the growing season and a low RGR during moisture stress.

2.2.3 Plant Height

Plant height showed significant positive associations with aerial biomass LAI, LAD, ears per plant and dry weight (Sharma *et al.*, 1991). Rawson *et al.*, (1996) observed that increasing mean temperature from 12 ^{0}C to 20 ^{0}C resulted in reduction in plant height by 16 %.

2.3 Yield and Yield Attributes

2.3.1 Biomass Weight

Sharma *et al.* (1991) observed significant positive association of aerial biomass with CGR, LAI, RGR, NAR, and LAD. Al-Katib and Paulsen (1990) measured various parameters and productivity in ten genotypes from major world wheat productivity regions under moderate (22 ^{0}C/17 ^{0}C day/night) and high (32 ^{0}C/27 ^{0}C day/night) temperature for two weeks as seedlings or from anthesis to maturity. They found that heat stress decreased mean total biomass by 32% and 15% in seedlings and maturing plants, respectively. Wheat plants grown in silt loan soil in a green house were subjected to day/night temperature of 15/10, 25/20 or 35/30 ^{0}C and high temperature exposure decreased shoots dry weight (Shah, 1992). For plants experiencing

a 3 ^{0}C increase in day and night temperatures relative to local mean temperatures, dry matter yields were reduced by 18% compared to control in wheat (Moot *et al.*, 1996). There are many other reports which emphasized that unfavorable temperature exposures upon sowing early or late by deviating from recommended time reduced total plant biomass in wheat (Singh and Rajat de, 1978, Randhawa *et al.*, 1981, Singh and Singh, 1985, Bhanu-prakash, 1997 and Chaturvedi *et al.*, 1985).

2.3.2 Tiller Numbers

Jain *et al.* (1974) suggested that number of ear bearing tillers at the time of harvest is an important yield component contributing to higher grain yield. Singh and Srivastava (1988) suggested that in wheat grain yield was dependent on tillers per plant during vegetative phase. Bansal and Sinha (1991) reported that maintenance of tillers was considered in maintaining the number of spikes across environment. Positive correlation of reproductive tillers number with grain yield have been observed (Sharma, 1995).

No significant influence on tillering by increasing day temperatures from 21 to 27 ^{0}C or night temperatures from 13 to 21 ^{0}C was observed (Campbell and Read, 1968). In the post earing phase temperature had great effect on number of ear/plant through number of tillers and percent fertile tillers. Owen, (1971) observed that plant given higher day temperatures continued to produce fertile tillers over a longer period. Mean temperature of 25 ^{0}C and above was always unfavorable for tillering and vegetative growth in October sown wheat (Bhardwaj, 1978). High temperatures during emergence to double ridge decreased number of spike bearing tillers (Shpiler and Blum, 1986 and Acevedo *et al.*, 1990). High mean temperature in early planting

compared to normal planting reduced tiller number in various wheat genotypes (Singh and Rajat de, 1978 and Randhawa *et al.*, 1981). Yield reduction of wheat under stress environment was attributed to reduced productive tillers per plant (Pal, 1992). Sharma, (1995) observed that reproductive tiller number was positively correlated with grain yield. Decrease in tiller number under field condition due to high temperature in wheat was also reported by Waines (1994).

2.3.3 Grain Number

One of the most important yield attributes affected by heat stress is grain number. In wheat the number of grains that develop in an ear is dependent on the number of viable florets that are formed and the effective fertilization of these after anthesis (Evans and Wardlaw, 1976). Grain number in wheat was reduced by warmer weather prevailing between floral initiation and anthesis (Owen, 1971). Good evidence has been found for a reduction in grain number per ear associated with high temperature during the stage of booting i.e., the stage of pollen and embryonic mother cell meiosis (Saini and Aspinall, 1982). High temperature upto 3 ^{0}C late in the development of the ear of wheat can result in a considerable reduction in the number of grain set at anthesis with the most sensitive stage at the time of pollen meiosis when the ear is still enclosed by the flag leaf sheath (Dawson and Wardlaw, 1989). Chaturvedi *et al.* (1985) suggested that tillers are affected more for number of spikelets and grains per spike and spike weight and grain weight per spike as compared to main shoot in late planting. Wardlaw *et al.* (1989) reported that reduction in grain yield associated with high temperature following anthesis results from variation in kernel size and not due to changes in kernel number.

Stone and Nicolas (1994) observed that short period of very high temperature (max > 35 ^{0}C) in the post anthesis period can significantly reduce grain yield in wheat. Most important yield component affecting yield variation among cultivars under heat stress is kernel number per spike. Under hot conditions heat tolerant cultivars sustain relatively more kernel per spike than heat susceptible cultivars (Shpiler and Blum, 1991). He and Rajaram, (1994) also observed that yield, seeds per spike, biomass and plant height were more thermosensitive than spike number per square meter, 1000 kernel weight and test weight. Shah *et al.* (1994) suggested that delayed sowing reduced grain number per ear, while Abdelghani *et al.* (1994) concluded that number of grains per ear were higher due to late sowing. Kelly *et al.* (1994) found that grain number per square meter was most closely correlated with grain yield followed by grain number per spike. Rawson *et al.* (1996) observed that increasing mean temperature from 12 ^{0}C to 20 ^{0}C resulted in 16% reduction in grain number per spike. Many researchers have concluded that the increases in yield and harvest index in wheat and barley have been almost entirely due to an increase in grain number per unit area, rather than to an increase in grain weight (Austin *et al.*, 1980, Mc Caig and DePauw, 1995 and Sayree *et al.*, 1996). Fisher, (1996) concluded that yield potential increase was correlated with higher harvest index and higher kernels per square meter and not with change in days to anthesis or biomass.

2.3.4 1000-Grain Weight

Chinoy (1947) compared grain weight per plant and 1000-grain weight of varieties grouped into eight classes according to flowering time, which ranged from 90-100 to 160-170, days with delay in flowering, grain developed

at increasingly higher temperatures and lower relative humidity, with the consequences that both 1000-grain weight and grain yield per plant diminished progressively. Asana and Williams (1965) concluded that increase in the grain weight was depressed beyond 25 ^{0}C due to increase in the respiration rate of grain. Kolderop, (1979) and Bhullar and Jenner (1985) reported that high temperature imposed 10 days after anthesis reduced yield of wheat through effects on individual kernel mass. Bhullar and Jenner (1983) also observed reduction in grain weight on brief warming of wheat ears. Grain weight was reduced due to reductions in individual grain weight and in grain number.

Wardlaw *et al.* (1989) observed that under controlled temperature conditions kernel weight was less susceptible to heat stress than kernel number. He and Rajaram (1994) also concluded that 1000 kernel weight was less sensitive than kernel number under heat stress. Delayed sowing decreased 1000-grain weight (Shah *et al.*, 1994 and Abdelghani *et al.*, 1995). Stone and Nicolas (1995) concluded that mature individual kernel mass was most sensitive to heat stress applied early in the grain filling and became progressively less sensitive through out grain filling. Hu and Fang (1995) found grain weight per ear was the main factor for improving grain weight per ear. Setter *et al.*, (1997) observed that stem carbohydrate which are accumulated before and during the early period after anthesis usually account for 10-30% of the stem dry weight in wheat, and in some cereals exposed to environmental stresses during grain filling this may contribute 70% or more of the grain weight.

2.3.5 Grain Yield

Late planting of wheat result in reduction of grain yield due to reduced crop duration and high temperature prevalent at the time of grain filling. Shah *et al.* (1994) observed that delayed sowing reduced grain yield. Crop sown in November showed 8-24% more grain yield than that sown in December (Singh *et al.*, 1995, Sarker and Torofder, 1992 and Singh and Verma, 1990). Asana and William (1965) observed that decrease in grain yield with increasing day temperature was remarkably uniform. The mean percentage reduction in yield for the 3 ^{0}C from 25 ^{0}C to 28 ^{0}C was 8.4 and 16.4 for 6 ^{0}C rise in temperature. Wardlaw and Wrigley (1994) concluded that in the high temperature range wheat yields decline approximately 3-4% for each 1 ^{0}C rise in average temperature above 15 ^{0}C. Anthesis and the end of grain filling period were advanced and grain and dry matter yield were reduced by 27 and 18% respectively in the plant experiencing a 3 ^{0}C increase in day and night temperatures (Moot *et al.*, 1996).

Guha-Sarkar *et al.* (2001) reported that early maturing genotypes like HD 2285, HD 2307, Sonalika, UP 2338, Lok 1 and C 306 showed better performance under high temperature stress conditions with less reduction in grain yield and have relatively higher grain growth rates with more tolerance to heat stress for most of the yield attributing characters. Guedira and Paulsen (2002) showed that high whole plant temperatures (30/30 ^{0}C shoot/root temperatures) accelerated linear grain growth in wheat but diminished the duration. Shoots as well as roots were responsive to thermal signals and elevated temperatures of both organs result in similar effects on the development and metabolism of the grain. Tahir and Nakata (2005) indicated

that heat stress negatively affected grain yield, its components and nitrogen remobilization while it increased total non structural carbohydrates remobilization because of the increasing demand for resources.

3. MATERIAL AND METHODS

To understand the mechanism of high temperature stress tolerance in wheat genotypes the present investigation was carried out in the Division of Plant Physiology, IARI, New Delhi, in field, pot culture and phytotron.

3.1 Climate and Weather

The Geographical Location of Delhi is 28^0-24' N Latitude and 72^0-12' E longitude in northern hemisphere at an altitude of 228.61 meters above mean sea level. Climatologically Delhi attains a semi dry sub-tropical climate with extremes for dry summer and cold winter. The mean maximum and minimum temperatures during hottest month (May) is about 41-42 ^{0}C. While the mean monthly minimum temperature in the coldest month (January) is as low as 5 ^{0}C. Thus, storms in summer and ground frost in winter are experienced quite often. The normal onset period of monsoon is in the third week of June and an annual rainfall of about 710 mm of which about 80 per cent is received during a short span of three months from mid June to mid September and the rest between October to May. The mean annual evaporation is about 850 mm, with mean daily evaporation ranging from as low as 2.2 mm in the month of January to as high as 16.0 mm in the month of June. Meteorological data on temperature, precipitation, relative humidity, were collected from Meteorological section, Division of Agriculture Physics, IARI, New Delhi.

3.2 Soil Characteristics

The topography of the field was fairly uniform with a gentle slope. The soil was sandy loam in texture, low in soil organic carbon (0.4%) available nitrogen (0.06%), phosphorous 32 kg/ha and medium in potassium 135 kg/ha. The soil was slightly alkaline in nature.

3.3 EXPERIMENT-1

The experiment was conducted in the Indian Agricultural Research Institute field, in the year of 2003-2004. Sowing done by seed drill at 10 cm deep in sandy loam soil at four lines for each genotypes, with three random replicates. Seeds were obtained from Division of Genetics, IARI. Sowing was done at three dates: normal (24/11/2003), late (22/12/2003) and very late (8/1/2004) in order to expose them to different temperature conditions (Fig. 1), and seed rate was 100 kg/ha in the normal sowing, while it was 125 kg/ha in the late and very late sowing. The following genotypes were used: rainfed (C 306, DL 153-2, HD 2781, HD 2865, HD 2866, HD 2868, HDR-77, HI 1500, RS 887, RS 888, K 8027 and PBW 175), irrigated (HD 2329, HD 2815, HD 2733, RS 827, RS 873, RS 854, PBW 343 and WR 544). Four irrigations were given during normal planting and five in the late and very late planting according to the water requirement. Fertilizers were applied as basal N, P, K i.e., 50, 60 and 40 kg/ha respectively and top doses were given N i.e., 50 kg/ha under normal planting and 75 Kg/ha under late and very late plantings.

3.3.1 Sampling

In each treatment, samples were collected from the upper most, healthy, fully expanded leaves for the first growth stages and subsequently the flag leaves was sampled.

3.3.2 Physiological Observations

3.3.2.1 Chlorophyll Estimation

Chlorophyll was estimated from all genotypes in three random replicate from the three planting date at three stages of growth (vegetative growth 30-35 days old seedling, anthesis and 15 days after anthesis). For chlorophyll estimation the methods suggested by Hiscox and Israelstam (1979) was followed. 50 mg of leaf materials from fully expanded leaf was weighed and incubated with 10 ml of Dimethyl sulphoxide (DMSO) at 65 ^{0}C for 4 hours to facilitate the extraction of chlorophyll into the solution. Absorbance were measured at 645 and 663 nm in spectronic 20 spectrophotometer and the amount of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were calculated using the formulae given by Arnon (1949)

Chlorophyll 'a' = 12.7 (A_{663}) - 2.68 (A_{645})

Chlorophyll 'b' = 22.9 (A_{645}) - 4.68 (A_{663})

Total chlorophyll = 22.2 (A_{645}) + 8.02 (A_{663})

3.3.2.2 Membrane Stability Index

Membrane stability index was estimated from all genotypes in three random replicates from the three planting date at three stages of growth (vegetative growth 30-35 day's old seedling, anthesis and 15 days after anthesis). A conductivity test was carried out to estimate heat tolerance as suggested by Deshmukh *et al.* (1991). 100 mg leaf sample was placed in a test tube containing 10 ml of double distilled deionized water. Tubes were incubated at 45 ^{0}C in water bath (York scientific Industries) for 40 min. Electrical conductivity of the solution was measured with the help of conductivity bridge of ELICO Pvt. Ltd. (CM 82T). The test tubes were kept at

100 ^{0}C for 10 min and after cooling conductivity were measured again. The method was standardized by repeated observations for uniform results. Per cent conductivity was determined by the following way:

$$\text{Per cent conductivity} = \frac{\text{C at } 45\ ^0\text{C}}{\text{C at } 100\ ^0\text{C}} \times 100$$

Where,

C= conductivity in mhos

3.3.2.3 Relative Water Content (RWC)

Relative water content was estimated in all the genotypes in three random replicates of the three plantings at three stages of growth (vegetative growth 30-35 day's old seedling, anthesis and 15 days after anthesis). It was determined by the method described by Barrs and Weatherley, (1962). 100 mg leaf material was taken and kept in double distilled water in a petridish for two hours to make the leaf tissue turgid. The turgid weights of the leaf materials were taken after carefully soaking the tissues between the two filter papers. Subsequently this leaf material was kept in a butter paper bag and dried in oven at 65 ^{0}C for 24 hours and their dry weights were recorded. The RWC was calculated by using the formula.

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

3.3.2.4 Leaf Water Transverse Relaxation Time (T_2)

T_2 value was determined at vegetative and anthesis stage and for that 3-4 fully expanded leaves were rolled into a cylinder (2 cm high) to fit tightly into a 10 cm diameter NMR sample tube for measurement of transverse relaxation time. The T_2 water protons were measured by Carr-purcell-

Meiboom-Gill (CPMG) method at 20 MHz with a Bruker NMR 120 pulsed NMR spectrometer. Each measurement had the following settings: data points 250, pulse separation- 0.5 ms, dummy echo 3, and scans 10, Gain was adjusted to maximize the signal to noise ratio. The data points were fitted mono-exponentially by the built- in Expspel program and average value of T_2 was obtained (Nagarajan *et al.*, 2005).

3.3.3 Growth Parameters

3.3.3.1 Plant Height

The length of main stem of five plants was measured in cm from ground level to the top.

3.3.3.2 Leaf Area per Plant

The leaf area per plant was recorded by automatic leaf area meter (LICOR-3000). The leaves of all plant collected and cleaned. Leaf area of only green leaves was measured.

3.3.3.3 Leaf Area Index

It is define as the area of assimilatory surface per unit land area.

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

3.3.3.4 Dry Matter Partitioning

The leaves, stems, ears of five plants were recorded and kept in oven at 80 ^{0}C for 48 hours. The dry weight of the leaves was recorded, averaged and expressed as leaf dry weight per plant. Total plant dry weight was recorded by adding the dry weight of leaves, stems and ears.

3.3.3.5 Crop Growth Rate (CGR)

Based on dry matter observation per unit land area at different growth stages CGR of crop calculated as follows:

$$CGR = \frac{w_2 - w_1}{(t_2 - t_1) \times A} \quad \text{.....g/m}^2\text{/day}$$

Where,

w_2 and w_1 are dry weights (g) recorded at time (days) t_2 and t_1 respectively. A is ground area in m^2.

3.3.3.6 Relative Growth Rate (RGR)

It is rate of increase in dry weight per unit of dry weight per unit of time and expressed as mg/mg/day. RGR was calculated by using the following formula:

$$RGR = \frac{\text{Log } w_2 - \text{log} w_1}{t_2 - t_1} \quad \text{.....g/g/day}$$

Where,

W_2 and w_1 = dry weight of plant in g at time t2 and t1 respectively.

t_2 and t_1 = time interval in days.

3.3.3.7 Net Assimilation Rate (NAR)

It is the rate of increase in dry matter per unit leaf area per unit of time (Watson, 1952).

$$NAR = \frac{w_2 - w_1}{t_2 - t_1} \times \frac{\log L_2 - \log L_1}{L_2 - L_1} \quad \text{.....mg/dm}^2\text{/day}$$

Where,

L_1 and W_1 = leaf area in dm^2 and dry weight of plant (mg) respectively at time t_1.

L_2 and W_2 = leaf area in dm^2 and dry weight of plant (mg) respectively at time t_2.

3.3.3.8 Flowering Date

Flowering date was determined by labeling five plants from each genotype with three random replicate in all the sowing dates and flowering date was recorded then mean value was calculated.

3.3.3.9 Growing Degree Days

The summation of mean temperature above a base value represents the growing degree days of thermal time.

$$GDD = \sum \frac{T\ max + T\ min}{2} - T\ b \quad \text{.....degree days}$$

Where,

T max and T min represents the daily maximum and minimum temperatures and T b is the base temperature and for wheat T b considered as 5 ^{0}C.

3.3.4 Yield and Yield Components

Number of seedlings from two meters rows was recoded after 3 weeks of sowing. Biomass and grain yield were recorded in three replicates from the two meters rows, in three random replicates at all plantings, grain weight and grain number per ear, 1000 grain weight were recorded on nine plants with three random replicates.

3.3.4.1 Harvest Index

It is the ratio of grain yield to biological yield per plant, expressed as per cent and calculated by using the formula:

$$HI\ (\%) = \frac{(\text{Grain yield} / m^2)}{(\text{Total biological yield} / m^2)} \times 100$$

3.3.4.2 Yield Stability Index

The yield stability index was calculated for grain yield as per the following formula:

$$YS = \frac{GY\ (H)}{GY\ (N)} \times 100$$

Where,

GY (H) : Grain yield under heat stress condition

GY (N) : Grain yield under normal condition.

3.3.4.3 Heat Use Efficiency

The heat use efficiency was calculated for dry matter production per m^2 at maturity and grain yield m^2 as per formula given by Rao *et al.*, 1999.

$$\text{Heat unit efficiency (biological yield)} = \frac{\text{Dry matter production } (g/m^2)}{GDD} \times 100 \quad \text{.....g.m}^{-2\,0}\text{. day}^{-1}$$

$$\text{Heat unit efficiency (grain yield)} = \frac{\text{Grain yield } (g/m^2)}{GDD} \times 100 \quad \text{.....g.m}^{-2\,0}\text{. day}^{-1}$$

3.3.4.4 Heat Susceptibility Index

Heat tolerance was viewed only in term of grain yield as suggested by (Fisher and Maurer, 1978).

$$S = \frac{(1\text{-}yh/yn)}{(1\text{-}Yh/Yn)}$$

Where,

y : represent the grain yield of specific genotype

Y : mean grain yield for the experiment

h : heat stress

n : normal temperature

3.4 EXPERIMENT-2

The experiment was carried out in the year of 2003-2004 in pot culture, Division of Plant Physiology, IARI. Earthen pots (heights 45 cm and diameter 30 cm) containing 10-12 kg sandy loam soil were used for this study and these were kept under natural light and atmospheric conditions. Farmyard manures (FYM) and soil was mixed in the ratio of 1:3 and used in each pot. NPK was applied in the ratio of 1:2:1 for each pot at the time of sowing. Sowing was done at three dates: normal (22/11/2003), late (22/12/2003), and very late (7/1/2004) at 10 cm deep in order to expose the genotypes to different temperature regimes (Fig. 2) and five seeds were kept in each pot. The following genotypes were used: PBW 343, PBW 175, HD 2865, HDR-77 and HD 2815. All the physiological and biochemical observations were recorded at vegetative stage (30-35 days old seedlings), anthesis and 15 days after anthesis.

3.4.1 Physiological and Biochemical Observations

3.4.1.1 Chlorophyll Estimation

As described in experiment 1.

3.4.1.2 Membrane Stability Index

As described in experiment 1.

3.4.1.3 Leaf Water Transverse Relaxation Time T_2

As described in experiment 1.

3.4.1.4 Leaf Water Potential

Water potential was determined at anthesis stage, 7 and 14 days after anthesis. Leaf water potential was measured on leaf sample using pressure chamber (S-pms Instruments, New Delhi, India) following the method described by Scholander *et al.*, 1964.

3.4.1.5 Photosynthetic Rate

It has been determined at three stages of growth, vegetative stage, anthesis and 15 days after anthesis. Net CO_2 assimilation rate of attached leaf blades of main shoot were measured using LI-6200 portable photosynthesis system of LI-COR, inc., Lincoln, Nebraska, USA (LI-6200 Primer: the instruction manual, 1989). The LI-6200 was operated in closed mode. Measurements were taken between 10 am to 12 noon. Photosynthetic rate were taken in three replicate and expressed as μ mol CO_2 used $m^{-2}.S^{1}$.

3.4.1.6 Antioxidant Enzymes Activity Assay

Superoxide dismutase and catalase enzymes were estimated at vegetative stage, anthesis and 15 days after anthesis from all genotypes, under all the plantings.

3.4.1.6.1 Superoxide Dismutase Estimation (SOD)

3.4.1.6.1.1 Preparation of Enzyme Extract

Enzyme extract for superoxide dismutase, was prepared by freezing 1 g leaf sample in liquid nitrogen to prevent protelytic activity followed by grinding the sample with 10 ml of extraction buffer (0.1 M phosphate buffer, PH 7.5 containing 0.5 mM EDTA) After filtration in 4 layers of cheesecloth it was centrifuged for 20 min at 15000 g and supernatant was used for estimation enzyme activity.

3.4.1.6.1.2 Reagents

1. **Methionine (200 mM):** 0.298g L-methionine was dissolved in double distilled water (ddw) and the volume was made up to 10 ml.
2. **Nitroblue tetrazolium chloride (NBT) (2.25 mM):** 0.0184 g NBT was dissolved in ddw and the volume was made up to 10 ml.
3. **EDTA (3.0 mM):** 0.0558 g EDTA was dissolved in ddw and the volume was made up to 50 ml.
4. **Riboflavin (60 µM):** 0.0023 g Riboflavin was dissolved in ddw and the volume was made up to 100 ml.
5. **Sodium carbonate (1.5 M):** 7.95 g Sodium carbonate was dissolved in ddw and the volume was made up to 50 ml.
6. **Phosphate buffer (100 mM, pH 7.8)**

Sol A: 6.80 g Potassium dihydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Sol B: 8.71 g Di-potassium hydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Buffer was prepared by mixing 8.5 ml sol. A and 91.5 ml of sol. B and final pH was adjusted with the help of pH meter.

3.4.1.6.1.3 Enzyme Assay

Superoxide dismutase activity was estimated by recording the decrease in optical density of formazone made by superoxide radical and nitro-blue tetrazolium dye by the enzyme (Dhindsa *et al.*, 1981). Three ml of the reaction mixture contained:

1. 13.33 mM methionine (0.2 ml of 200 mM)
2. 75 µM nitroblue tetrazolium chloride (NBT) (0.1 ml of 2.25 mM)

3. 0.1 mM EDTA (0.1 ml of 3 mM)
4. 50 mM phosphate buffer (pH 7.8) (1.5 ml of 100 mM)
5. 50 mM sodium carbonate (0.1 ml of 1.5 M)
6. 0.1 ml enzyme extract
7. 0.9 ml of ddw (to make final volume 3 ml)

Reaction was started by adding 2 mM riboflavin (0.1 ml) and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximum color, served as control. Then light was switched off and the tubes kept in dark to stop the reaction. A non-irradiated complete reaction mixture served as blank. Then absorbance was recorded at 560 nm and one unit of enzyme activity was taken as amount of enzyme, which reduced the absorbency reading to 50% in comparison with tubes lacking enzyme.

3.4.1.6.2 Catalase Estimation

3.4.1.6.2.1 Reagents

1. **Hydrogen peroxide (75 mM):** 775 µl of 30% H_2O_2 was dissolved in ddw and final volume was made to 100 ml.
2. **Phosphate buffer (100 mM, ph 7.0):**

Sol A: 6.8 g Potassium dihydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Sol B: 8.71 g Di-potassium hydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Phosphate buffer was prepared by mixing 39 ml of sol A and 61 ml of sol B and pH adjusted to 7.0.

3.4.1.6.2.2 Enzyme Assay

Catalase enzyme activity was estimated according to the method described by Aebi, (1984). Three ml of the following reaction mixture was prepared:

1. 50 mM Potassium phosphate buffer (1.5 ml of 100 mM buffer, pH 7.0)
2. 12.5 mM Hydrogen peroxide (0.5 ml of 75 mM H_2O_2)
3. 50 µl Enzyme extract
4. ddw to make final volume to 3 ml.

Reaction started by adding H_2O_2 and decrease in absorbance was recorded for 1 minute. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. The initial and final contents of hydrogen peroxide were calculated by comparing with a standard curve drawn with known concentrations of hydrogen peroxide. Enzyme activity was calculated as concentration of hydrogen peroxide reduced (initial reading-final reading = quantity of hydrogen peroxide reduced) per min. per mg protein.

3.4.2 Yield and Yield Component

Three pots were selected for recording yield parameters and data on total biomass per plant, grain yield per plant, grain number and grain weight per ear were taken from 9 plants randomly selected from these three pots and from the pooled grain yield per treatment, 1000 grain weight was recorded.

3.5 EXPERIMENT-3

This experiment was carried out in the pot culture in the year of 2004-2005. Earthen pots (heights 45 cm and diameter 30 cm) containing 10-12 kg sandy loam soil were used for this study and these were kept under natural light and atmospheric conditions. Farmyard manures (FYM) and soil are

mixed in the ratio of 1:3 was used in each pot. NPK was applied in the ratio of 1:2:1 for each pot at the time of sowing. Sowing was done at three dates: normal (9/11/2004), late (9/12/2004), very late (5/1/2005) at 10 cm deep in order to expose them to different temperature regimes (Fig. 3) and five seeds were kept in each pot. The following genotypes were used: PBW 343, PBW 175, HD 2865, HDR-77 and HD 2815. All the physiological and biochemical observations were recorded at vegetative stage (30-35 days old seedlings), anthesis and 15 days after anthesis.

3.5.1 Physiological and Biochemical Observations

3.5.1.1 Chlorophyll Estimation

As described in experiment 1.

3.5.1.2 Membrane Stability Index

As described in experiment 1.

3.5.1.3 Leaf Water Spin-Lattice Relaxation Time T_1

Leaf spin lattice relaxation time T_1 was measured at 20 MHz and at ambient temperature of 25 ^{0}C by pulsed NMR instrument (Bruker Minispec PC-20) using 90 ^{0}-τ-90 ^{0} pulse sequence (Farrar and Becker, 1971).

3.5.1.4 Photosynthetic Rate

As described in experiment 2

3.5.1.5 Respiration Rate

For respiration rate measurement LI-6200 portable photosynthesis system was used like photosynthetic rate measurement except that the chamber was covered with a black cloth. It has been taken in three replicate and expressed as μ mol O_2 released $m^{-2}s^{-1}$.

3.5.1.6 Leaf Water Potential

As described in experiment 2.

3.5.1.7 Stem Reserve Mobilization

Ten plants were selected for each genotype from three pots. Out of ten plants, five plants were selected and main shoot were totally defoliated and tillers also were removed, while the other five plants tillers were removed but main shoot was not defoliated. Defoliation was done eight days after anthesis and after maturity individual spike was harvested and weight was taken and stems reserve mobilization was calculated as followed:

$$\text{Stem reserve mobilization} = \frac{\text{Weight of spike from defoliated plant}}{\text{Weight of spike from non defoliated plant}} \times 100$$

3.5.1.8 Antioxidant Enzymes Estimation

3.5.1.8.1 Superoxide Dismutase (SOD)

As described in experiment 1.

3.5.1.8.2 Ascorbate Peroxidase

3.4.1.8.2.1 Preparation of Enzyme Extract

Enzyme extract for ascorbate peroxidase was prepared by freezing 1g leaf sample in liquid nitrogen to prevent protelytic activity followed by grinding the sample with 10 ml of extraction buffer (0.1 M phosphate buffer, PH 7.5 containing 0.5 mM EDTA and 1 mM ascorbic acid). After filtration in 4 layers of cheesecloth it was centrifuged for 20 min at 15000 g and supernatant was used for estimation enzyme activity. However estimation was done in the same day to avoid any reduction in enzyme activity.

3.5.1.8.2.2 Reagents

1. **Ascorbic acid (3.0 mM):** 0.0265 g Ascorbic acid was dissolved in ddw and the volume was made up to 50 ml.
2. **EDTA (3.0 mM):** 0.0558 g EDTA was dissolved in ddw and the volume was made up to 50 ml.
3. **Hydrogen peroxide (1.5 mM):** 16 µl standard hydrogen peroxide (30%) was dissolved in ddw and the volume was made up to 100 ml.
4. **Phosphate buffer (100 mM, pH 7.0):**

Sol A: 6.80 g Potassium dihydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Sol B: 8.71 g Di-potassium hydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Buffer was made by mixing 39 ml of sol. A and 61 ml of sol. B and pH was adjusted to 7.0 with pH meter.

3.5.1.8.2.3 Enzyme Assay

Ascorbate peroxidase was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm (Nakano and Asada, 1981). Three ml of reaction mixture contained:

1. 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM)
2. 0.5 mM ascorbic acid (0.5 ml of 3.0 mM)
3. 0.1 mM EDTA (0.1 ml of 3.0 mM)
4. 4. 0.1 mM H_2O_2 (0.1 ml of 3.0 mM)

0.1 ml enzyme 6. 0.7 ml ddw (to make the final volume to 3.0 ml)

The reaction was started with the addition of 0.1 ml of hydrogen peroxide. Decrease in absorbance for a period of 30 seconds was recorded at

290 nm in an UV-visible spectrophotometer (model M 36, Beckman, Ca., USA). Activity is expressed by calculating the decrease in ascorbic acid content by comparing with a standard curve drawn with known concentrations of ascorbic acid.

3.5.1.8.3 Glutathione Reductase Estimation (GR)

3.5.1.8.3.1 Preparation of Enzyme Extract

Same as SOD

3.5.1.8.3.2 Reagents

1. **5,5- Dithiobis- (2-nitobenzoic acid)(DTNB) (3.0 mM):** 0.011 g of DTNB was dissolved in phosphate buffer and the volume was made up to 10 ml with phosphate buffer (10 mM pH 7.5).
2. **Oxidized glutathione (GSSG) (20 mM):** 0.319 g of GSSG was dissolved in ddw and the volume was made up to 2.5 ml.
3. **NADPH (2.0 mM):** 0.017 g of NADPH was dissolved in ddw and the volume was made up to 10 ml.
4. **Phosphate buffer (200 mM, pH 7.5):**

Sol A: 6.80 g Potassium dihydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Sol B: 8.71 g Di-potassium hydrogen phosphate was dissolved in ddw and the volume was made up to 500 ml.

Buffer was prepared by mixing 15 ml of sol.A and 85 ml sol.B and pH was adjusted to 7.5 with the help of pH meter.

3.5.1.8.3.3 Enzyme Assay

Glutathione reductase was assayed by using the method suggested by (Smith *et al.*, 1988). The reaction mixture contained:

1. 66.67 mM potassium phosphate buffer

   (1 ml of 0.2 m buffer containing 1 mM EDTA)

2. 0.5 mM DTNB in 0.01 M potassium phosphate buffer

   (pH 7.5) (0.5 ml of 3.0 mM)

3. 66.67 µM NADPH (0.1 ml of 2.0 mM)

4. 666.67 µM GSSG (0.1 ml of 20 mM)

5. 0.1 ml enzyme extract

6. Distilled water to make up a final volume of 3.0 ml.

Reaction was started by adding 0.1 ml of 20.0 mM GSSG (Oxidized glutathione), and increase in absorbance was recorded at 412 nm. The activity is expressed as total absorbency (ΔA_{412}) per mg protein per min.

3.5.1.8.4 Catalase Assay

3.5.1.8.4.1 Reagents

1. **Phosphate buffer (0.1 M, pH 7.0):**

Sol A: 0.1 M Di-potassium hydrogen phosphate prepared by dissolving 1.74g in ddw and final volume was made up to 100 ml.

Sol B: 0.1 M Potassium dihydrogen phosphate prepared by dissolving 1.74g in ddw and final volume was made up to 100 ml.

Buffer was prepared by mixing 61 ml sol. A and 39 ml sol. B and final pH was adjusted to 7.0.

2. **Hydrogen peroxide (6 mM):** A stock solution of 6 mM H_2O_2 was prepared by dissolving 1.03 ml of 30% hydrogen peroxide in 100 ml of ddw. From this stock solution 6 ml was pipette out and diluted to 100 ml using ddw to get desired concentration.

3. **Titanium reagent:** Titanium reagent was prepared as described by Teranishi *et al.*, (1974). One gram of titanium dioxide and 10g potassium sulphate were mixed and digested with 150 ml of concentrated sulphuric acid for 2 hours on a hot plate. The digested mixture was cooled and diluted to 1.5 liter with ddw and used as titanium reagent.

3.5.1.8.4.2 Enzyme Assay

Catalase was assayed by measuring the disappearance of H_2O_2 according to Teranishi *et al.* (1974). Reaction mixture (3 ml) consisted of 1 ml of 6 mM H_2O_2 and 1.9 ml of 0.1 M phosphate buffer (pH 7.0) were kept in test tubes, and the reaction was initiated by adding 0.1 ml of diluted enzyme extract. The reaction was stopped after 5 minutes by addition of 4 ml of titanium reagent, which also forms color complex with residual H_2O_2. Reaction mixture without enzyme served as control and developed maximum color with titanium reagent. Aliquots were centrifuged at 10,000 g for 10 minutes and absorbance of supernatant was recorded at 415 nm in spectrophotometer. The residual H_2O_2 content in samples were computed with the help of standard curve.

3.5.1.8.5 Peroxidase Estimation

3.5.1.8.5.1 Reagents

1. **Phosphate buffer (100 mM, pH 6.1)**

Sol A: 6.8 g Potassium dihydrogen phosphate dissolved in ddw and the volume made up to 500 ml.

Sol B: 8.71 g Di-potassium hydrogen phosphate dissolved in ddw and the volume made up to 500 ml.

Buffer was prepared by mixing 15 ml of sol A. and 85 ml of sol B. and pH adjusted to 6.1 with the help of pH meter.

2. **Hydrogen peroxide (12 mM):** 124 µl of 30% H_2O_2 Dissolved in ddw and final volume made to 100 ml.

3. **Guaiacol (96 mM):** 1075 µl of analytical grade guaiacol was dissolved in ddw and final volume made to 100 ml.

3.5.1.8.5.2 Enzyme Assay

Enzyme was assayed according to the method described by Castillo *et al.* (1984)

Three ml of the reaction mixture was prepared as followed:

1. Phosphate buffer (50 mM, ph 6.1): 1.0 ml of 100 mM
2. Guaiacol (16 mM) : 0.5 ml of 96 mM
3. H_2O_2 (2 mM) : 0.5 ml of 12 mM
4. Enzyme extract : 0.1 ml
5. ddw : 0.9 ml to make final volume to 3.0 ml

Absorbance due the formation of tetra-guaiacol was recorded at 470 nm and enzyme activity was calculated as per extinction coefficient of its oxidation product. Enzyme activity is expressed as µmol tetra-guaiacol formed per min. per mg protein.

3.5.1.8.6 Total Soluble Protein Estimation

Soluble protein in the leaves was determined by the method given by Bradford, 1976.

3.5.1.8.6.1 Reagents

Dye concentrate: 100 mg of Coomassie brilliant blue dye (G-250) was dissolved in 50 ml of 95 per cent ethanol, 100 ml concentration of orthophoric

acid was added to it. Final volume was made to 200 ml with ddw. The solution was kept in amber color bottle at 4 ^{0}C.

3.5.1.8.6.2 Procedure

1. **Extraction:** 500 mg fresh leaf material was ground using pestle and mortar with 3 ml of cold extraction buffer (60 mM Tris HCl), pH 6.8. The extract was centrifuged at 15000 rpm for 15 minutes. The supernatant was made to 5 ml with extraction buffer. It was used as stock crude protein.
2. **Estimation:** the concentrated dye was diluted with distilled water (1 dye: 4 distilled water). Filtered with Whatman No. 1 paper if precipitation occurs. To 5 ml of diluted dye, 30 ml of leaf crude protein and 80 ml water was added. The contents were vortexed and absorbance was measured at 595 nm after 5 minutes.

3.5.2 Yield and Yield Component

Three pots were selected for collecting yield parameters and data on total biomass per plant, grain yield per plant, grain number and grain weight per ear were taken from 9 plants randomly selected from these three pots and from the pooled grain yield per treatment, 1000 grain weight was recorded.

3.5.3 Grain Growth Rate

Absolute growth rate (AGR) of individual grains from middle spikelets were computed between sampling periods of 5, 10, 15, 20, 25, 30, 35,40 days after anthesis. Absolute growth rate was computed according to the following formula (Gregory, 1926 and Watson, 1952).

$$\text{Absolute growth rate (AGR)} = \frac{W_2 - W_1}{t_2 - t_1}$$

Where

W_1 and W_2 are the dry weights of single grain sample (mg) collected at times t_1 and t_2 (in days) respectively.

3.6 EXPERIMENT-4

This experiment was conducted in the growth chamber (Phytotron facility), Indian Agricultural Research Institute, New Delhi. In one growth chamber, temperature was maintained as 23-18 ^{0}C day/night (control) through out the experiment while in the other chamber the temperature was raised after maximum tillering (45-50 day-old seedlings) to 35/25 ^{0}C day/night (heat stress treatment). In both growth chamber 14 hours light and 10 hours dark were given, and maximum light intensity was 21 Klux and minimum was 0.2 Klux and relative humidity was maintained at 65-70%. Two wheat genotypes were selected, heat stress tolerant (C 306) and heat stress susceptible (PBW 343). Thirty pots (6 inch size) of each genotype were kept in each chamber. The composition of pot media was 2:1:1 (Coco-Coir peat: Vermiculite: Quartz revier sand). Pots were autoclaved at 20 PSI for three hours and half before sowing. Seeds of both genotypes were treated with mercuric chloride 0.01% for 10 second, then rinsed by 70% alcohol for 10 second then washed three times with ddw then dried in blotting papers before sowing and 6 seeds were kept in each pot. Deinonized water were given in each pots till germination , ¼ Hogland and Arnon (1950) solution were given till 2-3 leaf stage then full Hogland and Arnon solutions were given.

3.6.1 Physiological and Biochemical Observations

Chlorophyll content, membrane stability index, leaf water spin–lattice relaxation time T_1, photosynthetic rate, respiration rate, SOD, APX, Catalase, GR and POX were estimated at three stages of growth (after one week of heat stress treatment, at anthesis stage and at 15 days after anthesis) as described in experiment 1 and 3. Water potential was determined at anthesis stage, 7 and 14 days after anthesis.

3.6.1.1 Hydrogen Peroxide Content:

Hydrogen peroxide content (H_2O_2) was estimated according to method described by Mukherjee and Choudhari (1983).

3.6.1.1.1 Reagents

1. **Titanium reagent:** prepared as described in experiment 3.
2. **Liquid ammonia solution**
3. **Acetone solution**
4. **Sulphuric acid (1 M)**: 55 ml of concentrated sulphuric acid were dissolved in ddw and the volume was made up to 1 liter.

3.6.1.1.2 Procedure

0.5 g leaf sample was homogenized in 10 ml of cold acetone. The homogenate was filtered through Whatman No. 1 filter paper. To the whole extract 4 ml of titanium reagent was added followed by 5 ml of concentrated ammonium solution to precipitate hydroperoxide-titanium complex. After centrifugation for 5 minutes at 10,000 g, the supernatant was discarded and precipitate was dissolved in 1 M sulphuric acid. It was re-centrifuged to remove undissolved material and absorbance was recorded at 415 nm

against blank. Concentration of H_2O_2 was determined using standard curve plotted with known concentration of H_2O_2.

A standard curve of H2O2 was prepared by taking 0.1, 0.2.....1 µmol of H_2O_2 in ddw from a stock solution of 1 mM H_2O_2. The hydroperoxide-titanium complex is formed by adding 4 ml titanium reagent and 5 ml of concentrated ammonia and processed as described for sample. Absorbance values were plotted against concentration and linear curve was drawn and used for obtaining the concentrations of the sample.

3.6.1.2 Rubisco Enzyme Assay

Rubisco enzyme was estimated according to the method described by Fair and Cresswell, (1973)

3.6.1.2.1 Reagents

1. **Tris buffer (150 mM, pH 7.5):** Tris (300 mM) solution was prepared by dissolving 3.63 g Tris salt in ddw, pH of the solution was adjusted to 7.5 with 0.2 N HCl and the final volume was made up to 200 ml.
2. **Grinding media:** grinding media consisted of 0.112 g EDTA (10 mM) and 0.046 g glutathione-reduced (5 mM) in final volume of 30 ml phosphate buffer.
3. **Radioactive sodium bicarbonate solution (100 mM+ 5 mCi/10 ml):** Sodium bicarbonate (84 mg) was dissolved in ddw and 5 mCi of radioactive bicarbonate was added. The final volume was made up to 10 ml.
4. **Ribulose diphosphate solution (40 mM):** 2 mg ribulsoe 1,5 bisphosphate sodium salt (Sigma Chemioal Co., USA) was dissolved in 5 ml of ddw.

5. **Magnesium chloride (300 mM):** 3.05 g magnesium chloride was dissolved in ddw and volume was made to 50 ml.
6. **Ethylene diamine tetrachloroacetic acid (EDTA) (10 mM):** 0.186g EDTA was dissolved in ddw and volume was made to 50 ml.
7. **Scintillation fluid:** 4 g of PPO (2,5 diphenyloxazole) and 100 mg of POPOP (4,4-bis(2,4 methyl 1,5 phenyl oxazolyl-benzene) were dissolved in 1000 ml of toluene.

3.6.1.2.2 Enzyme Extraction

500 mg freshly cut leaf material was ground in a chilled mortar and pestle with 10 ml of pre-cooled grinding media and acid washed sand. The extract is squeezed through three layers of cheesecloth and the filtrate was centrifuged at 10,000 g for 15 minutes at 4 ^{0}C in a refrigerated centrifuge. The supernatant was used as source of the enzyme.

3.6.1.2.3 Enzyme Assay

Enzyme assay was done in the glass scintillation vials, in a final volume of 0.57 ml. The reaction mixture consisted of the following:

1.	Enzyme extract	:	100 µl
2.	$NaH^{14}CO_3$ solution (20 umol+10 µCi)	:	200 µl of 100 mM+5 mCi
3.	Ribulose bisphosphate (0.2 µmol)	:	50 µl of 40 mM
4.	Magnesium chloride (3.0 µmol)	:	10 µl of 300 mM
5.	EDTA (0.1 µmol)	:	10 µl of 10 mM
6.	Tris HCl buffer, pH 7.5 (30.0 µmol)	:	200 µl of 150 mM

A blank was run simultaneously having all the reagents and boiled enzyme. The reaction mixture was incubated for 10 minutes and the reaction was stopped by adding 0.2 ml of 6.0 N acetic acid, which also removed the

excess of unfixed bicarbonates. The solution was evaporated to dryness in an oven at 50 ^{0}C. 10 ml of scintillation fluid was added to the vials and the counts per minute were recorded in a Packard Tri-Carb Scintillation Spectrometer. Results were expressed as (µmol $^{14}CO_2$ fixed mg^{-1} protein min^{-1}).

For calculation of activity, blank vials containing only ^{14}C-bicarbonate (10 µCi/20 µmol $NaH^{14}CO_3$) are used and their reading in cpm (counts per minutes) recorded along with samples.

Molarity of carrier bicarbonate/(cpm of sample/cpm of blank)

$$\text{Rubisco activity µmol } {}^{14}CO_2 \text{ fixed mg}^{-1} \text{ protein min}^{-1} = \frac{\text{Molarity of carrier bicarbonate}}{\text{cpm of sample/cpm of blank)}} \times \frac{1}{\text{f.w. of sample}}$$

$$\times \frac{\text{Total volume of extract}}{\text{Vol. of enzyme used}} \times \frac{1}{\text{incubation time (min)}} \times \frac{1}{\text{protein content}}$$

3.6.2 Molecular Analysis of gene expression

The effect of heat stress on the expression of small and large subunits of Rubisco was studied through RT-PCR and northern analysis was used to study the effect of heat stress on the expression of D1 protein, oxygen evolving complex, thylakoid ascorbate peroxidase and HSP 101. For that, tissues were sampled at three stages viz., one week after heat stress treatment (50- day-old seedlings), anthesis and 15 days after anthesis.

3.6.2.1 RNA Isolation

All glass and plasticwears to be used for RNA work were washed thoroughly with detergent, rinsed with double distilled water and soaked overnight in 0.02% DEPC treated water. Glass and plasticwears were dried in oven at 80 ^{0}C and sterilized by autoclaving at 121 ^{0}C for 15 min.

For RNA isolation TRIZOL Reagent (Invitrogen, Life Technologies) was used. 1 g leaf sample was ground in liquid nitrogen, 10 ml of trizol was added, and after vortexing, samples were incubated at room temperature for 5 min. 2 ml of chloroform was added and after vigorous shaking samples were incubated at room temperature for 10 min. Tubes were centrifuged at 12000 g for 10 min at 4 ^{0}C. Aqueous phase was collected and 5 ml of isopropanol was added. After incubation at for 10 min at room temperature, tubes were centrifuged at 12000 g for 10 min at 4 ^{0}C. The pellet was retained and washed with 70% ethanol (prepared with DEPC treated water). After centrifugation at 10000 g for 10 min at 4 ^{0}C, the liquid was discarded and the pellet was air dried and then dissolved in 300 µl DEPC treated water. Quality and quantity of RNA was checked by electrophoresis of an aliquot in 1% TBE gel and by spectro-photometric measurement at 260 and 280 nm.

3.6.2.2 Primers Design

Primers for Rubisco small and large sbunits, D1 protein, oxygen evolving complex, ascorbate peroxidase and HSP 101 of wheat were designed based on cDNA sequence information available at the NCBI site (http:// www.ncbi.nlm.nih.gov). The primer sequences (5'-3') are given below:

rbcS-F	:	CGT GAT GGC TTC GTC GGC TAC
rbcS-R	:	CTG AAT GCG ATG AAG CTG AC
RbcL-F	:	CGC CTC ATG GTA TCC AAG TTG
RbcL-R	:	CGA TTA GCT GCT GCA CCA GGT G
PsbO-F	:	GAC CTT CGA CGA GAT CCA GA
PsbO-R	:	CCC TGG ATC TTG ACA TCC TT
PSII32-F	:	GGA AGC TGC ATC TGT TGA TG
PSII32-F	:	CAA GGT TAG CAC GGT TGA TG
HSP101-F	:	CTT CGA CGA GGT TGA GAA GG

HSP101-R : CTG GAT CAG GAT GTC GGA CT

tAPX-F : GGC ATG ATT CGG GTA CAT ATG
tAPX-R : CCT GGT CCT CTG CGT ACT TC

Primers were dissolved in RNAse free water and 10 uM working stock of each primer was prepared.

3.6.2.3 RT-PCR

For RT-PCR /Qiagen One step RT-PCR Kit/ was used. Reaction mixtures were prepared as follows:

1. 10 µl 5x Qiagen One Step RT-PCR Buffer
2. 2 µl dNTP mix 400 µM of each dNTP
3. 0.6 µM of each forward and reverse primer
4. 2 µl Qiagen One Step RT-PCR Enzyme Mix
5. RNase inhibitor (potential) 5-10 units/reaction
6. RNase free water and (variable volume used)
7. Template RNA 2 µg/reaction

Total volume 50 µl

Thermal cycle conditions as follow:

1. 50 ^{0}C for 30 min (reverse transcription reaction take place)
2. 95 ^{0}C for 15 min (activation HotStar Taq DNA Polymerase and inactivation Reverse Transcriptases)
3. 94 ^{0}C for 1 min
4. 58 ^{0}C for 1 min
5. 72 ^{0}C for 1 min
6. Cycle repeated 34 times from step number 3 to step number 5.
7. 72 ^{0}C for 10 min (for Final extension)

cDNA was checked at 1.4% agarose gel. A negative control (PCR reaction with RNA as template) and a positive control (genomic DNA as template) were included to ascertain DNA contamination or PCR conditions were not adversely affecting our results.

3.6.2.4 Northern Blotting

3.6.2.4.1 Preparation of Buffer Solutions

1. 10 X MOPS:

41.2 g	3-(N-morpholino) propane sulphonic acid
10.9 g	Sodium acetate 3 hydrate
3.7 g	EDTA disodium salt

Salts were dissolved in 800 ml of nuclease free water (DEPC treated water) pH adjusted to 7.0 with NaOH and final volume was made to 1 liter. Solutions was sterilized by filtration and stored at room temperature in amber colored bottle to protect MOPS against oxidization by light.

2. 10 X RNA loading dye buffer:

0.25%	Bromophenol blue
0.25%	Xylene cyanol FF
1 mM	EDTA
50%	Glycerol

Dissolved in 1 ml of DEPC treated water

3. 20 X SSC:

175.3 g of NaCl and 88.2 g of sodium citrate were dissolved in 800 ml of DEPC- treated water. pH was adjusted to 7.0 with a few drops of 10 N NaOH and final volume was made up to 1 liter. Dispensed into aliquots and sterilized by autoclaving.

4. Prehybridization /Hybridization Buffer:

SDS	70 g
1 M Phosphate buffer pH 7.2	500 ml
0.5 M EDTA disodium salt	20 ml

Volume was made up to 1 liter with DEPC treated water.

5. 1.2% MOPS Gel:

250 ml gel was prepared by melting 3 g agarose in 210 ml of DEPC treated water and 25 ml of 10X MOPS and 15 ml formaldehyde. Gel was covered from light to prevent oxidation of MOPS by light. 3 µl ethidium bromide was added when solution cooled to 65 ^{0}C, and poured into gel casting tray. Agarose gel assembly was washed with DEPC treated water and kept overnight before use. Equal volume of RNA loading dye (2X) and the RNA were mixed and 30 µl was loaded into wells of gel. 1 X MOPS was used as running buffer. Electrophoresis was carried out at a constant 55 V. for 4 hours.

3.6.2.4.2 RNA Loading Mixture:

10 X MOPS	3 µl
Formaldehyde	5.5 µl
Formamide	10 µl
RNA	11.5 µl

8 µl high ranges RNA ladder was used as marker and polythene strip was used to mark the positions of the bands of the marker. Before loading the sample a pre- run for 20 min at 50 V was done. Before loading, RNA samples were denatured at 65 ^{0}C for 10 min.

3.6.2.4.3 Northern Transfer and Hybridization

The gel was placed in a clean, sterile DEPC treated glass tray and rinsed in DEPC treated water for 15 min with gentle shaking. The gel was then soaked in 20X SSC for 45 min at room temperature. The RNA was transferred from gel to nylon membrane by wet capillary blotting using 20X SSC as the transfer buffer. A glass plate covered by a wick of Whatman No. 3 Paper was dipped in a tank of 20X SSC. After 45 min the gel was placed upside down on the wick and above this the nylon membrane and 3-5 pieces of Whatman No. 3 cut to the same size of gel were placed in the same order. 5-7 cm thick stack of blotting paper was placed on the assembly. This was followed by a glass plate and ½ kg weight. The blotting was allowed to continue for 16-18 hours. The membrane was dried and RNA was immobilized either through baking at 80 ^{0}C for 2 hour or by UV crossing linking at 245 nm for 1 to 5 min the membrane was dipped in 2X SSC, air dried and stored in dessicator until further use.

Prehybridization was done in glass tubes by putting the nylon membrane with 35 ml of prehybridization buffer at 60 ^{0}C in hybridization oven for 3 hours at 15-20 rpm. After adding 50 µl of radio probe hybridization was continued for 18 hours at 80 ^{0}C. At the end of hybridization membrane was washed with (2X SSPE + 0.1% SDS) for 5 min at room temperature then with (1X SSPE + 0.1% SDS) for 10 min at 65 ^{0}C then with (0.5X SSPE + 0.1% SDS) for 10 min at 65 ^{0}C. The washed membrane was kept with an X-ray film in a cassette and stored at 80 ^{0}C for 2-5 days before developing.

3.6.2.4.3.1 Probe Preparation:

DNA used for probe preparation was amplified by PCR using primer specific to D1 protein, oxygen evolving complex, ascorbate peroxidase and HSP 101 of wheat.

50 µl PCR reaction mixture was prepared using (Qiagen Taq PCR Kit) as follows:

10 µl 10 X PCR buffer

25 mM $MgCl_2$ (variable)

2 µl dNTP mix (10 mM each)

1.5 µl Forward Primer 0.5 µM

1.5 µl Reverse Primer 0.5 µM

0.5 µl Taq DNA Polymerase (2.5 units/reaction)

DEPC treated water (variable)

2 µl genomic DNA (20 ng/µl)

Total volume was made 50 µl.

PCR conditions were:

1- 4 min at 94 ^{0}C (DNA denaturation)

2- 1 min at 94 ^{0}C

3- 1 min at 55 ^{0}C (for ascorbate peroxidase, D1 protein) and 56 ^{0}C for (oxygen evolving complex and HSP 101) (primer annealing)

4- 1 min at 72 ^{0}C (primer extension)

5- Steps 2-4 repeated for 34 times

6- 10 min at 72 ^{0}C (final extension)

3.6.2.4.4 Probe Labeling:

For probe labeling HexaLabel™ DNA labeling kit (Fermentas/USA) was used. 100 ng DNA template (6 µl) and 5X buffer (10 µl) and deionized water (24 µl) were mixed in an eppendroff tube and kept in boiling water for 10 min. After snap cooling for 10 min in ice, 2 µl deionized water, 3 µl -dCTP mix ,2 µl radiolabel dCTP and 1 µl Klenow fragment was added and kept at 37 ^{0}C for 10 min then 4 µl of dNTP mix was added and after 5 min the reaction terminated by adding 1 µl of 0.5 M EDTA.

20 X SSPE:

175.3 g of NaCl and 27.6 g of $NaH_2PO_4.H_2O$ and 7.4 g of EDTA were dissolved in 800 ml of DEPC-treated water. pH was adjusted to 7.4 with NaOH and final volume was made to 1 liter with DEPC-treated water, dispensed into aliquots, then sterilized by autoclaving.

3.6.2.4.5. Developing X-ray Film

3.6.2.4.5.1 Reagents

Preparation of developer: (5 g of powder A and 75.12 g of powder B) were dissolved by vigorous shaking In 500 ml ddw and stored in a dark colored bottle.

Preparation of Fixer: 132.22 g of fixer was dissolved In 500 ml ddw and stored in dark colored bottle.

The X-ray film was immersed in developer solution for 5 min rinsed in water and fixed by immersing in fixer solution for 5 min. Developing and fixing were carried out under red light.

3.7 Statistical analysis

Analysis of variance was performed on the data at each stage (Panse and Sukhatme, 1967). Critical difference (CD) values were calculated at the 5 per cent probability level.

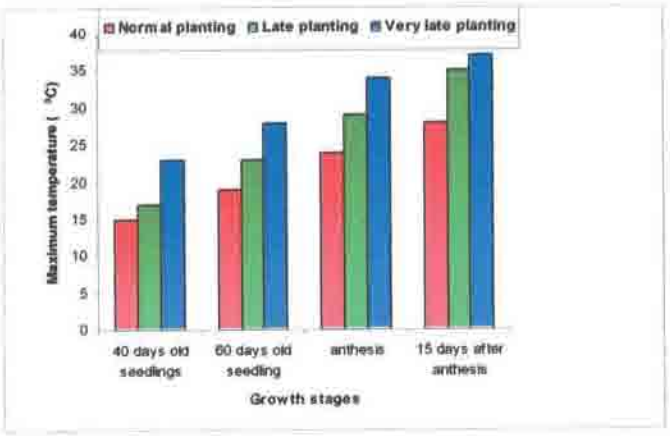


Fig.1. Maximum temperature (^{0}C) at 40 and 60 days old seedling, anthesis and 15 days after anthesis under normal, late and very late planting in the year of 2003-2004

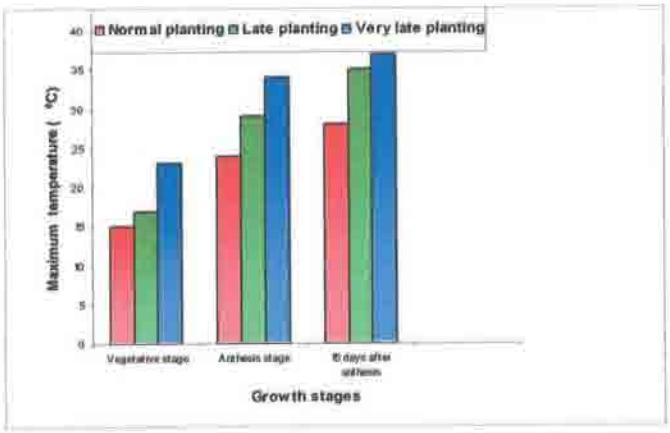


Fig. 2. Maximum temperature (^{0}C) at vegetative stage, anthesis and 15 days after anthesis under normal, late and very late planting in the year of 2003-2004

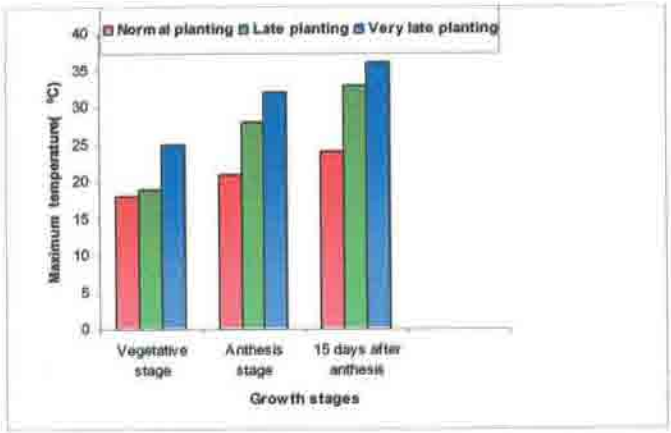


Fig. 3. Maximum temperature (^{0}C) at vegetative stage, anthesis and 15 days after anthesis under normal, late and very late planting in the year of 2004-2005

4. RESULTS

4.1 EXPERIMENT-1

Delay of wheat sowing has significant effect on the temperatures experienced by plants at different stages of growth. During normal sowing plants experienced the temperature of 15, 19, 24 and 27 ^{0}C, under late planting 17, 23, 30 and 34 ^{0}C and under very late planting 23, 28, 34 and 37 ^{0}C at 40 and 60 days after sowing (DAS), anthesis and 15 days after anthesis (DAA), respectively. Temperature recorded at the time of sampling under normal, late and very late planting showed the significant increase in temperature under late and very late planting compared to normal planting particularly at reproductive stage (Fig. 1).

4.1.1 Physiological Parameters

4.1.1.1 Chlorophyll Content

Highest chlorophyll-a content under normal and late planting in all genotypes was recorded at anthesis stage, while under very late planting highest chlorophyll-a content was recorded at 15 days after anthesis. However, significant reduction in chlorophyll-a content was recorded under late and very late planting compared to normal planting in all genotypes at all stages of growth. Irrigated genotypes showed higher chlorophyll-a content

during vegetative stage than rainfed genotypes under all plantings, while at anthesis and 15 days after anthesis rainfed genotypes showed higher chlorophyll-a content than irrigated genotypes under all planting dates (Table 1). Highest chlorophyll-b content was recorded at 15 days after anthesis in all genotypes under all plantings. Significant reduction in chlorophyll-b content was observed under late and very late planting compared to normal planting at all growth stages. At vegetative stage and 15 days after anthesis highest chlorophyll-b content was recorded under normal planting in irrigated genotypes and no significant differences were recorded under late and very late planting between irrigated and rainfed genotypes. At anthesis stage highest chlorophyll-b content was recorded under normal and late planting in irrigated genotypes, while under very late planting highest chlorophyll-b content was recorded in rainfed genotypes (Table 2). Under normal, late and very late planting highest chlorophyll a:b ratio was recorded at vegetative stage, anthesis and at 15 days after anthesis respectively. Significant increase in a:b ratio was recorded under late and very late planting compared to normal at all stages of growth. Irrigated genotypes showed higher a:b ratio compared to rainfed genotypes under late and very late planting conditions (Table 3).

Highest total chlorophyll content in all the genotypes was recorded at 15 days after anthesis, under normal, late and very late plantings. Irrigated genotypes showed greater total chlorophyll content under normal planting, while under late and very late planting highest chlorophyll content was recorded in rainfed genotypes at all growth stages (Table 4). The per cent of reductions in chlorophyll content under late planting compared to normal

Table 1. Chlorophyll a content (mg g^{-1} fresh weight) in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	1.40	1.26	1.11	2.14	1.94	1.88	2.11	2.04	1.84
DL-153-2	1.55	1.25	1.20	2.18	1.97	1.62	2.08	1.97	1.45
HD2781	1.42	1.12	1.08	2.10	1.86	1.68	2.47	2.30	2.08
HD2865	1.40	1.21	1.13	2.18	2.02	1.87	2.13	1.96	1.84
HD2866	1.52	1.36	1.25	2.12	2.01	1.74	2.15	1.98	1.76
HD2868	1.49	1.25	1.14	2.18	2.09	1.92	1.97	1.92	1.83
HDR-77	1.61	1.35	1.25	2.24	2.14	1.84	2.27	2.19	1.74
HI1500	1.75	1.54	1.33	2.25	2.17	1.49	2.09	1.97	1.87
RS887	1.49	1.24	1.19	2.12	2.08	1.68	1.98	1.91	1.78
RS888	1.43	1.25	1.19	2.15	1.98	1.84	1.89	1.92	1.86
K8027	1.28	1.31	1.24	1.98	1.93	1.59	1.85	1.82	1.75
PBW175	1.46	1.40	1.24	2.19	2.02	1.87	2.14	1.93	1.86
Mean	**1.48**	**1.30**	**1.20**	**2.15**	**2.02**	**1.75**	**2.09**	**1.99**	**1.81**
Irrigated									
HD2329	1.49	1.30	1.25	2.19	1.91	1.30	2.05	1.87	1.65
HD2815	1.66	1.51	1.31	2.30	2.15	1.76	1.94	1.85	1.77
HD2733	1.56	1.28	1.06	2.07	1.94	1.81	2.24	2.11	1.84
PBW343	1.54	1.26	1.16	2.05	1.97	1.61	1.96	1.84	1.78
WR544	1.38	1.32	1.22	1.85	1.64	1.56	1.61	1.54	1.51
RS827	1.57	1.37	1.28	2.28	2.11	1.42	2.22	2.11	1.87
RS873	1.61	1.44	1.29	2.30	1.99	1.41	2.09	1.82	1.62
RS854	1.55	1.41	1.25	1.90	1.74	1.58	1.93	1.59	1.50
Mean	**1.55**	**1.36**	**1.23**	**2.12**	**1.93**	**1.56**	**2.01**	**1.84**	**1.69**
Total mean	1.51	1.32	1.21	2.14	1.98	1.67	2.06	1.93	1.76
CD at 5%	0.066	0.139	0.242	0.032	0.059	0.102	0.034	0.080	0.139

Table 2. Chlorophyll b content (mg g^{-1} fresh weight) in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	0.69	0.60	0.52	0.96	0.81	0.76	0.99	0.94	0.81
DL-153-2	0.62	0.52	0.50	0.81	0.67	0.50	1.00	0.92	0.60
HD2781	0.75	0.56	0.51	1.61	1.33	1.00	1.19	1.08	0.44
HD2865	0.88	0.81	0.71	0.93	0.83	0.76	1.76	1.48	0.76
HD2866	0.80	0.76	0.57	1.25	0.83	0.79	1.26	1.00	0.63
HD2868	0.91	0.62	0.52	1.07	0.99	0.52	1.28	1.09	0.86
HDR-77	0.67	0.61	0.55	1.15	0.91	0.67	0.93	0.84	0.49
HI1500	0.61	0.60	0.50	0.83	0.68	0.35	1.08	0.98	0.67
RS887	0.77	0.65	0.53	1.14	0.88	0.84	0.78	0.63	0.38
RS888	0.79	0.65	0.54	0.99	0.90	0.81	1.32	1.11	0.60
K8027	0.72	0.52	0.40	0.94	0.87	0.50	1.20	1.01	0.42
PBW175	0.91	0.62	0.59	1.41	1.39	0.54	1.53	1.22	0.87
Mean	**0.76**	**0.63**	**0.54**	**1.08**	**0.93**	**0.68**	**1.19**	**1.03**	**0.63**
Irrigated									
HD2329	0.77	0.32	0.30	1.19	0.74	0.42	1.27	0.90	0.38
HD2815	0.79	0.58	0.53	1.16	1.00	0.76	1.57	1.46	0.84
HD2733	0.96	0.92	0.62	1.54	1.10	1.07	1.15	1.01	0.39
PBW343	0.92	0.81	0.41	1.56	0.70	0.36	1.55	0.85	0.54
WR544	0.88	0.79	0.72	1.09	0.98	0.56	1.52	1.17	0.78
RS827	0.71	0.67	0.33	1.19	0.90	0.25	1.27	0.62	0.44
RS873	0.61	0.47	0.42	1.23	1.05	0.57	1.45	0.80	0.69
RS854	0.78	0.74	0.65	1.50	1.31	0.44	1.42	1.61	1.11
Mean	**0.80**	**0.66**	**0.50**	**1.31**	**0.97**	**0.55**	**1.40**	**1.05**	**0.65**
Total mean	0.78	0.64	0.52	1.17	0.95	0.63	1.27	1.04	0.64
CD at 5%	0.037	0.116	0.202	0.025	0.034	0.060	0.031	0.086	0.149

Table 3. Chlorophyll a:b ratio in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	2.03	2.10	2.13	2.23	2.40	2.47	2.13	2.17	2.27
DL-153-2	2.50	2.40	2.40	2.69	2.94	3.24	2.08	2.14	2.42
HD2781	1.89	2.00	2.12	1.30	1.40	1.68	2.08	2.13	4.73
HD2865	1.59	1.49	1.60	2.34	2.43	2.46	1.21	1.32	2.42
HD2866	1.90	1.79	2.19	1.70	2.42	2.20	1.71	1.98	2.79
HD2868	1.64	2.02	2.19	2.04	2.11	3.69	1.54	1.76	2.13
HDR-77	2.40	2.21	2.27	1.95	2.35	2.75	2.44	2.61	3.55
HI1500	2.87	2.57	2.66	2.71	3.19	4.26	1.94	2.01	2.79
RS887	1.94	1.91	2.25	1.86	2.36	2.00	2.54	3.03	4.68
RS888	1.81	1.92	2.20	2.17	2.20	2.27	1.43	1.73	3.10
K8027	1.78	2.52	3.10	2.11	2.22	3.18	1.54	1.80	4.17
PBW175	1.60	2.26	2.10	1.55	1.45	3.46	1.40	1.58	2.14
Mean	**1.95**	**2.07**	**2.23**	**1.99**	**2.17**	**2.59**	**1.75**	**1.94**	**2.88**
Irrigated									
HD2329	1.94	4.06	4.17	1.84	2.58	3.10	1.61	2.08	4.34
HD2815	2.10	2.60	2.47	1.98	2.15	2.32	1.24	1.27	2.11
HD2733	1.63	1.39	1.71	1.34	1.76	1.69	1.95	2.09	4.72
PBW343	1.67	1.56	2.83	1.31	2.81	4.47	1.26	2.16	3.30
WR544	1.57	1.67	1.69	1.70	1.67	2.79	1.06	1.32	1.94
RS827	2.21	2.04	3.88	1.92	2.34	5.68	1.75	3.40	4.25
RS873	2.64	3.06	3.07	1.87	1.90	2.47	1.44	2.28	2.35
RS854	1.99	1.91	1.92	1.27	1.33	3.59	1.36	0.99	1.35
Mean	**1.93**	**2.05**	**2.47**	**1.62**	**1.99**	**2.81**	**1.43**	**1.75**	**2.62**
Total mean	1.88	1.92	2.13	1.83	2.09	2.63	1.61	1.86	2.76
CD at 5%	0.226	0.561	0.972	0.096	0.115	0.199	0.471	0.117	0.203

Table 4. Total chlorophyll content (mg g^{-1} fresh weight) in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	2.09	1.86	1.63	3.10	2.75	2.71	3.10	2.98	2.65
DL-153-2	2.17	1.77	1.70	2.91	2.73	2.12	3.08	2.89	2.05
HD2781	2.17	1.68	1.59	3.71	3.19	2.68	3.66	3.38	2.52
HD2865	2.28	2.02	1.84	3.11	2.85	2.63	3.89	3.44	2.60
HD2866	2.32	2.12	1.82	3.37	2.84	2.53	3.41	2.98	2.39
HD2868	2.40	1.87	1.66	3.25	3.08	2.44	3.25	3.01	2.69
HDR-77	2.28	1.96	1.80	3.39	3.05	2.51	3.20	3.03	2.23
HI1500	2.36	2.14	1.83	3.08	2.85	1.84	3.17	2.95	2.54
RS887	2.26	1.89	1.72	3.26	2.96	2.52	2.76	2.54	2.16
RS888	2.22	1.90	1.73	3.14	2.88	2.65	3.21	3.03	2.46
K8027	2.00	1.83	1.64	2.92	2.80	2.09	3.05	2.83	2.17
PBW175	2.37	2.02	1.83	3.60	3.41	2.41	3.67	3.15	2.73
Mean	**2.24**	**1.92**	**1.73**	**3.24**	**2.95**	**2.43**	**3.29**	**3.02**	**2.43**
Irrigated									
HD2329	2.26	1.62	1.55	3.38	2.65	1.72	3.32	2.77	2.03
HD2815	2.45	2.09	1.84	3.46	3.15	2.52	3.51	3.31	2.61
HD2733	2.52	2.20	1.68	3.61	3.04	2.88	3.39	3.12	2.23
PBW343	2.46	2.07	1.57	3.61	2.67	1.97	3.51	2.69	2.32
WR544	2.26	2.11	1.94	2.94	2.62	2.12	3.13	2.71	2.29
RS827	2.28	2.04	1.61	3.47	3.01	1.67	3.49	2.73	2.31
RS873	2.22	1.91	1.71	3.53	3.04	1.98	3.54	2.62	2.31
RS854	2.33	2.15	1.90	3.40	3.05	2.02	3.35	3.20	2.61
Mean	**2.35**	**2.02**	**1.73**	**3.43**	**2.90**	**2.11**	**3.41**	**2.89**	**2.34**
Total mean	2.29	1.96	1.73	3.31	2.93	2.31	3.33	2.97	2.40
CD at 5%	0.081	0.147	0.255	0.051	0.128	0.222	0.080	0.101	0.288

planting was 15, 11.4 and 10.9% at vegetative, anthesis and at 15 days after anthesis respectively. Under very late planting at vegetative stage, anthesis and at 15 days after anthesis the per cent of reduction was 31.3, 30.3 and 28.1% respectively. Greater reduction in total chlorophyll content was recorded under late and very late plantings compared to normal planting in irrigated genotypes than in rainfed genotypes. In irrigated genotypes per cent of reductions in total chlorophyll content under late planting compared to normal at anthesis and 15 days after anthesis were 15.2 and 15% respectively, while in the rainfed genotypes the reductions were 8.9 and 8.2% respectively. Under very late planting per cent of reductions in total chlorophyll content compared to normal planting in irrigated genotypes at vegetative, anthesis and 15 days after anthesis were 26.5, 38.4 and 31.3% respectively, while in rainfed genotypes the reduction were 22.8, 25 and 26% respectively. Among the twenty genotypes, minimum reduction in total chlorophyll content under late and very late planting compared to normal was recorded in C 306, HDR-77, HI 1500 and K 8027 (rainfed), WR 544, HD 2815 and RS 854 (irrigated). Maximum reduction was recorded in HD 2781, HD 2868 (rainfed), PBW 343, HD 2329 (irrigated) under late plantings.

4.1.1.2 Membrane Stability Index

Membrane stability index (MSI) decreased with growth of the crop in all the plantings. There was significant reduction in MSI under late and very late planting compared to normal. Reductions in MSI under late planting compared to normal were 21.8, 19.3 and 11.2% at vegetative stage, anthesis and 15 days after anthesis respectively, while under very late planting per cent reductions compared to normal planting were 29.3, 27.2 and 18.9%

Table 5. Membrane stability index (%) in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	71.0	56.0	52.0	73.4	57.9	49.7	60.2	56.7	49.3
DL-153-2	73.8	64.0	53.0	70.4	62.7	56.3	55.5	49.2	46.7
HD2781	77.5	48.2	46.0	74.7	48.7	43.3	58.5	48.7	41.9
HD2865	69.4	42.6	38.5	55.5	44.8	39.5	46.8	44.4	38.5
HD2866	74.6	46.3	39.7	71.0	44.6	38.7	57.3	45.2	40.1
HD2868	75.8	46.6	39.1	70.1	48.0	34.0	57.9	43.7	37.5
HDR-77	74.3	67.0	58.0	69.8	59.7	53.7	58.7	51.3	48.8
HI1500	76.8	67.7	59.8	71.7	62.7	57.7	57.8	47.2	36.1
RS887	73.4	46.7	40.3	69.1	46.3	40.2	57.4	45.4	38.8
RS888	72.5	46.7	39.9	70.2	47.7	41.1	57.3	46.2	37.5
K8027	76.0	68.3	58.7	67.6	48.1	43.1	52.4	47.7	39.8
PBW175	74.5	47.5	42.1	67.7	48.0	43.1	59.6	44.8	38.5
Mean	**74.1**	**54.0**	**47.2**	**69.3**	**51.6**	**45.0**	**56.6**	**47.5**	**41.1**
Irrigated									
HD2329	75.7	45.7	34.4	57.0	42.0	31.7	64.1	42.4	29.7
HD2815	74.3	47.6	43.6	68.1	49.2	43.3	66.8	47.7	41.2
HD2733	75.3	53.5	43.2	67.6	45.7	34.1	57.0	44.2	38.4
PBW343	74.2	44.5	31.3	70.7	46.3	30.3	49.8	36.6	24.7
WR544	72.1	64.3	57.7	67.8	49.9	39.7	53.4	46.8	35.4
RS827	76.1	41.0	39.4	70.8	44.8	35.0	55.1	44.4	34.6
RS873	78.9	48.8	38.0	69.5	47.0	35.6	59.4	44.9	37.7
RS854	71.6	59.5	48.7	69.4	43.6	39.9	58.8	43.4	31.7
Mean	**74.8**	**50.6**	**42.0**	**67.6**	**46.0**	**36.2**	**58.0**	**43.8**	**34.2**
Total mean	74.4	52.6	45.1	68.6	49.3	41.4	57.2	46.0	38.3
CD at 5%	2.138	2.994	5.186	6.590	3.667	6.352	2.544	2.724	4.719

respectively. However irrigated crop showed more reduction in MSI compared to rainfed crop under late and very late plantings compared to normal. There were 24.2, 21.5 and 14.3% reductions in MSI in irrigated genotypes at vegetative, anthesis and 15 days after anthesis under late planting compared to normal planting, while rainfed genotype showed 20.2, 17.7 and 9.1% reductions respectively. Under very late planting irrigated genotypes showed 32.8, 31.4 and 23.9% reductions compared to normal planting, while rainfed genotypes showed 26.9, 24.2 and 15.5% reductions at vegetative stage, anthesis and at 15 days after anthesis respectively (Table 5). Genotypes HDR-77, HI 1500, DL153-2, K 8027 and C306 (rainfed) and HD 2815 and WR 544 (irrigated) showed minimum reduction in MSI and better thermostability under late and very late plantings compared to other genotypes. HD 2781, HD 2868 and RS 887 (rainfed) and PBW 343, HD 2329 (irrigated) showed highest reduction in MSI under late plantings.

4.1.1.3 Relative Water Content

More relative water content (RWC) in all genotypes was recorded at vegetative stage under normal, late and very late plantings. Irrigated genotypes showed higher RWC than rainfed ones under normal planting, while under late and very late plantings RWC was higher in rainfed genotypes compared to rainfed ones at all stages of the growth (Table 7). The reduction in RWC under late planting compared to normal at vegetative stage, anthesis and at 15 days after anthesis was 7.1, 5.9 and 4.4% respectively, while under very late planting the reduction was 13.1, 11 and 8.9% respectively. In irrigated genotypes per cent reductions in RWC under late planting compared to normal at vegetative stage, anthesis and 15 days after anthesis was 9, 7.6

Table 6. Relative water content (%) in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting at vegetative, antheis and 15 days after anthesis

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	81.6	75.6	71.4	74.8	70.2	67.9	74.9	71.4	68.5
DL-153-2	82.0	76.7	72.8	77.6	72.1	70.6	77.8	73.6	67.3
HD2781	74.9	68.2	63.8	75.9	67.3	62.4	72.4	67.8	65.5
HD2865	80.0	74.4	68.1	74.4	70.4	67.5	73.7	70.8	63.4
HD2866	81.5	73.7	68.2	78.7	73.3	68.8	75.1	70.2	64.6
HD2868	80.3	70.2	66.8	79.2	74.5	70.3	74.9	71.5	64.2
HDR-77	81.3	78.9	73.8	79.3	76.9	74.8	75.1	72.8	71.4
HI1500	82.5	80.6	79.0	80.7	78.9	75.5	79.2	75.2	73.9
RS887	80.7	76.7	71.3	80.4	76.7	67.7	77.9	74.4	71.3
RS888	80.5	73.3	70.1	79.9	73.0	65.5	75.0	71.7	67.7
K8027	80.2	75.3	72.3	78.0	75.4	73.6	75.1	71.7	67.6
PBW175	80.0	72.3	65.8	77.7	70.7	64.7	74.2	70.5	67.5
Mean	**80.5**	**74.6**	**70.3**	**78.0**	**73.3**	**69.1**	**75.4**	**71.8**	**67.7**
Irrigated									
HD2329	89.3	71.1	65.0	79.3	67.9	60.4	75.1	67.7	60.2
HD2815	82.9	76.7	72.2	75.4	71.3	66.3	71.4	68.7	66.8
HD2733	80.6	72.4	67.7	79.5	70.6	64.7	74.3	70.7	65.5
PBW343	87.0	77.8	63.7	80.7	71.7	62.8	76.6	68.0	62.0
WR544	79.7	76.4	74.7	77.7	72.7	70.5	74.0	68.4	63.8
RS827	85.2	70.3	62.3	79.3	71.7	61.9	80.6	73.7	68.3
RS873	87.2	81.7	64.7	80.2	72.6	65.8	80.0	75.3	65.7
RS854	85.0	78.6	71.1	80.4	73.2	68.7	71.5	67.3	66.4
Mean	**84.6**	**75.6**	**64.5**	**79.0**	**71.5**	**65.1**	**75.4**	**69.9**	**64.8**
Total mean	82.2	75.0	69.1	78.5	72.5	67.5	75.4	71.1	66.6
CD at 5%	0.840	1.922	3.061	2.433	2.969	2.339	1.109	2.004	2.472

and 5.5% respectively, while in the rainfed genotypes the decrease was 5.8, 4.8 and 3.6% respectively. Under very late planting per cent reductions in RWC compared to normal in irrigated genotypes at vegetative stage, anthesis and 15 days after anthesis was 20.1, 13.9 and 10.6%, while in rainfed genotypes it was 10.2, 8.9 and 7.7% respectively. Minimum reductions in RWC under late and very late planting were recorded in HDR-77, HI 1500, K 8027 and C 306 (rainfed) and WR 544, HD 2815 and RS 854 (irrigated). Greater reduction in RWC was recorded under late plantings in HD 2781 and HD 2868 (rainfed) and PBW 343 and HD 2329 (irrigated).

4.1.1.4 Leaf Water Transverse Relaxation Time (T_2)

Maximum leaf water transverse relaxation time (T_2) was recorded at vegetative stage in all genotypes under all plantings. Leaf water T_2 values at vegetative stage under normal, late and very late planting were 332, 293 and 255 ms respectively, while at anthesis stage it was 222, 206 and 182 ms respectively. However there was significant reduction in transverse relaxation time (T_2) under late and very late planting compared to normal planting at vegetative and anthesis stages. Irrigated genotypes showed more reduction in T_2 compared to rainfed genotypes at vegetative and anthesis stages under late and very late planting compared to normal (Table 7). Per cent reductions in T_2 in irrigated genotypes under late planting compared to normal were 13.7 and 9.5% at vegetative and anthesis stages respectively, while rainfed genotypes showed 10.4 and 5.5% respectively. In irrigated genotypes under very late planting per cent reductions compared to normal planting at vegetative and anthesis stages were 27.6 and 22.9% respectively, while rainfed genotypes showed 20.2 and 14.3% respectively. Among the

Table 7. Transverse relaxation time T_2 (ms) in twenty (irrigated and very late planting at vegetative, anthesis stages

Genotypes	Vegetative stage			Anthesis stage		
	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed						
C306	308	286	276	210	201	180
DL-153-2	385	343	318	231	218	192
HD2781	350	310	248	200	189	174
HD2865	303	265	236	206	187	176
HD2866	284	225	183	196	182	176
HD2868	320	273	236	225	213	184
HDR-77	324	310	287	194	188	179
HI1500	290	270	260	205	187	172
RS887	367	326	287	202	199	170
RS888	330	308	269	199	186	171
K8027	295	263	244	218	207	185
PBW175	313	284	244	218	208	185
Mean	**322**	**289**	**257**	**209**	**197**	**179**
Irrigated						
HD2329	345	255	228	246	231	166
HD2815	339	329	287	254	246	231
HD2733	317	284	235	222	202	167
PBW343	383	331	245	246	210	168
WR544	327	307	275	228	213	197
RS827	329	258	213	241	216	171
RS873	387	307	258	290	233	199
RS854	341	318	264	201	194	186
Mean	**346**	**299**	**251**	**241**	**218**	**186**
Total mean	332	293	255	222	206	182
CD at 5%	11.42	13.25	17.48	6.66	10.60	16.35

genotypes C 306, HDR-77 and HI 1500 (rainfed) and HD 2815, RS 854 and WR 544 (irrigated) showed better performance and less reduction in T_2 values under late and very late planting compared to normal planting. HD 2781 and HD 2868 (rainfed) and HD 2329 and PBW 343 (irrigated) showed greater reductions under late plantings.

4.1.2 Growth parameters

4.1.2.1 Leaf Area Index

More leaf area index (LAI) was recorded at anthesis stage under all sowings in all the genotypes. Mean values at 40, 60 DAS, anthesis and 15 days after anthesis under normal planting were 2.12, 3.07, 5.41 and 3.93. However, there was significant reduction in LAI under late and very late plantings compared to normal planting. Mean values of LAI under late planting were 1.13, 2.08, 3.71 and 2.04 respectively, while under very late planting values were 0.65, 0.99, 1.56 and 0.89 respectively (Table 8). Under late planting the per cent reduction compared to normal planting at 40, 60 DAS, anthesis and 15 days after anthesis was 46.7, 32.2, 27.4 and 48% respectively, while under very late planting the per cent reduction compared to normal planting was 69.4, 67.8, 69.5 and 77.3% respectively. The per cent reductions under late planting compared to normal planting in irrigated genotypes at 40, 60 DAS, anthesis and 15 days after anthesis were 49.5, 36.2, 32.9 and 54.3% respectively, while rainfed genotypes showed 44.6, 29.2, 23.2 and 43.3% respectively. Under very late planting the per cent reduction compared to normal planting in irrigated genotypes was 73, 74, 75.8 and 81.1% respectively, while in rainfed genotypes the per cent reduction was 66.7, 63, 64.8 and 74.5% respectively.

Genotypes	40 days old seedling			60 days old seedling			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed												
C306	2.19	1.31	0.78	2.89	2.08	1.17	5.5	3.74	2.08	3.63	2.85	1.03
DL-153-2	1.90	1.13	0.69	2.45	2.01	1.49	4.76	4.26	1.76	3.26	3.17	0.89
HD2781	1.81	1.13	0.63	3.17	2.15	0.94	5.08	3.33	1.42	3.76	1.96	0.55
HD2865	1.98	1.13	0.64	2.82	2.18	0.90	4.76	3.61	1.50	3.51	2.31	0.91
HD2866	2.25	1.07	0.61	3.02	2.06	1.10	5.53	3.64	1.75	3.87	1.56	0.87
HD2868	2.25	1.11	0.58	3.55	2.35	1.32	5.53	3.22	1.87	3.88	2.12	0.96
HDR-77	2.56	1.54	1.08	2.43	1.93	1.21	4.04	3.73	1.81	4.54	2.82	1.21
HI1500	2.32	1.15	0.77	3.66	2.49	1.45	5.79	4.53	1.97	4.73	2.71	1.34
RS887	1.98	1.00	0.64	2.75	1.98	0.76	4.86	3.44	1.54	3.66	0.9	0.87
RS888	1.73	1.01	0.46	2.95	1.83	0.87	4.60	4.15	1.48	3.81	1.87	0.75
K8027	1.67	1.12	0.71	2.63	2.22	1.12	4.31	3.66	1.96	3.51	1.99	1.54
PBW175	2.26	1.09	0.70	3.23	1.90	0.81	4.32	4.04	1.64	3.91	1.84	0.81
Mean	**2.08**	**1.15**	**0.69**	**2.96**	**2.10**	**1.10**	**4.92**	**3.78**	**1.73**	**3.84**	**2.18**	**0.98**
Irrigated												
HD2329	1.60	0.87	0.53	3.26	2.12	1.07	4.90	3.47	1.20	3.79	1.75	0.67
HD2815	2.04	1.37	0.75	3.17	2.14	1.08	4.79	3.60	1.54	4.04	2.81	1.23
HD2733	2.27	1.07	0.65	3.44	2.54	1.56	5.42	3.79	2.15	3.98	1.89	0.74
PBW343	2.21	1.03	0.43	3.46	2.03	0.62	5.38	3.44	0.86	4.14	1.55	0.72
WR544	2.35	1.25	0.78	2.92	2.06	0.72	5.43	3.34	1.83	3.66	1.24	0.98
RS827	1.96	0.94	0.47	3.04	1.87	0.44	5.38	3.59	0.86	3.90	1.99	0.56
RS873	2.96	1.08	0.54	3.84	1.65	0.66	6.33	3.48	0.97	5.16	2.14	0.55
RS854	2.08	1.21	0.57	2.75	2.11	0.57	5.36	4.15	1.01	3.79	1.48	0.68
Mean	**2.12**	**1.10**	**0.59**	**3.24**	**2.07**	**0.84**	**5.37**	**3.61**	**1.30**	**4.06**	**1.86**	**0.77**
Total mean	2.12	1.13	0.65	3.07	2.08	0.99	5.11	3.71	1.56	3.93	2.04	0.89
CD at 5%	0.030	0.026	0.021	0.047	0.109	0.189	0.028	0.174	0.302	0.062	0.106	0.184

4.1.2.2 Crop Growth Rate

Under normal planting highest crop growth rate (CGR) values were recorded at anthesis stage, while under late and very late planting the values were higher at 15 days after anthesis. Mean CGR values under normal planting at vegetative stage, anthesis and at 15 days after anthesis were 1.537, 3.895 and 3.682 (g/m^2/day) respectively, under late planting 0.807, 2.484 and 2.497 (g/m^2/day) respectively, and under very late planting 0.431, 1.040 and 1.176 (g/m^2/day) respectively (Table 9). There was significant reduction in CGR under late and very late planting compared to normal planting. The per cent reduction under late planting compared to normal planting at vegetative stage, anthesis and 15 days after anthesis was 47.5, 36.2 and 32.2% respectively, while under very late planting the per cent reduction was 72, 57.9 and 68% respectively. More reductions in CGR was recorded in irrigated genotypes compared to rainfed genotypes at all stages of growth under late and very late planting compared to normal. The per cent reduction in irrigated genotypes under late and very late plantings compared to normal was 56.4, 46.5 and 40.6% and 79.1, 64.4 and 71.1% respectively, while in rainfed genotypes the reduction was 39.3, 28.7 and 26% and 65.4, 53.1 and 65.8% respectively under late and very late plantings.

4.1.2.3 Relative Growth Rate

Under normal planting higher relative growth rate (RGR) was recorded at vegetative stage, while under late and very late plantings the values were higher at anthesis stage. The mean RGR values under normal planting at vegetative stage, anthesis and at 15 days after anthesis were 0.286, 0.219 and 0.026 (g/g/day) respectively, under late planting 0.244, 0.34 and 0.068

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	1.16	0.80	0.45	4.14	2.97	2.35	3.74	2.57	1.63
DL-153-2	1.14	0.77	0.43	3.79	3.32	1.89	3.79	3.02	1.37
HD2781	1.08	0.45	0.26	4.84	3.62	1.63	4.04	2.82	1.21
HD2865	1.56	0.99	0.58	3.99	2.52	1.75	3.59	3.02	1.30
HD2866	1.81	1.29	0.48	3.25	2.32	1.51	3.35	2.32	1.03
HD2868	0.86	0.56	0.26	3.98	2.71	1.72	3.98	3.01	1.05
HDR-77	1.08	0.95	0.62	4.18	2.88	2.30	4.08	2.88	1.68
HI1500	1.33	0.81	0.56	3.87	2.90	1.84	2.97	1.90	1.25
RS887	1.54	0.88	0.41	4.13	2.63	1.71	3.83	2.99	1.02
RS888	2.02	0.85	0.45	3.46	2.05	1.80	3.14	2.04	0.99
K8027	1.21	0.83	0.65	2.42	2.07	1.52	3.02	2.47	1.19
PBW175	1.48	0.72	0.48	3.78	2.65	1.46	3.48	2.77	1.01
Mean	**1.36**	**0.82**	**0.47**	**3.82**	**2.72**	**1.79**	**3.58**	**2.65**	**1.23**
Irrigated									
HD2329	1.88	0.54	0.40	4.65	1.87	0.97	4.35	2.17	0.99
HD2815	1.77	0.97	0.46	4.12	2.41	1.69	3.82	2.91	0.84
HD2733	1.62	0.64	0.23	3.57	2.08	1.41	3.57	2.48	1.14
PBW343	2.25	0.75	0.46	4.83	2.79	1.38	4.68	2.35	1.08
WR544	1.73	1.14	0.57	3.94	1.67	1.83	3.74	2.20	1.43
RS827	1.73	0.75	0.29	3.76	2.17	1.24	3.46	1.88	0.99
RS873	1.69	0.69	0.27	3.58	2.01	1.44	3.38	1.66	1.00
RS854	1.73	0.80	0.33	3.62	2.15	1.46	3.62	2.55	1.37
Mean	**1.80**	**0.78**	**0.38**	**4.01**	**2.14**	**1.43**	**3.83**	**2.28**	**1.10**
Total mean	1.54	0.81	0.43	3.90	2.48	1.04	3.68	2.50	1.18
CD at 5%	0.03	0.13	0.13	0.12	0.16	0.22	0.12	0.18	0.23

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	0.278	0.251	0.239	0.274	0.378	0.331	0.037	0.073	0.057
DL-153-2	0.253	0.239	0.213	0.240	0.393	0.328	0.024	0.054	0.053
HD2781	0.273	0.206	0.171	0.257	0.318	0.278	0.022	0.056	0.036
HD2865	0.263	0.251	0.232	0.160	0.345	0.274	0.019	0.075	0.045
HD2866	0.302	0.287	0.258	0.149	0.297	0.267	0.025	0.074	0.055
HD2868	0.250	0.238	0.203	0.295	0.359	0.306	0.037	0.086	0.059
HDR-77	0.309	0.281	0.259	0.280	0.381	0.342	0.028	0.084	0.077
HI1500	0.245	0.228	0.216	0.115	0.342	0.315	0.018	0.075	0.062
RS887	0.307	0.244	0.211	0.213	0.315	0.287	0.012	0.062	0.051
RS888	0.316	0.286	0.236	0.098	0.310	0.261	0.012	0.076	0.034
K8027	0.286	0.274	0.220	0.165	0.349	0.316	0.028	0.096	0.085
PBW175	0.240	0.236	0.202	0.189	0.379	0.278	0.019	0.073	0.043
Mean	**0.277**	**0.252**	**0.222**	**0.203**	**0.347**	**0.299**	**0.023**	**0.074**	**0.055**
Irrigated									
HD2329	0.320	0.231	0.210	0.278	0.315	0.296	0.024	0.052	0.036
HD2815	0.283	0.241	0.209	0.250	0.354	0.324	0.029	0.091	0.061
HD2733	0.293	0.217	0.203	0.245	0.354	0.287	0.026	0.047	0.030
PBW343	0.335	0.234	0.204	0.283	0.323	0.302	0.031	0.034	0.026
WR544	0.293	0.262	0.218	0.232	0.387	0.318	0.037	0.077	0.064
RS827	0.286	0.235	0.208	0.228	0.268	0.231	0.025	0.056	0.035
RS873	0.294	0.213	0.210	0.209	0.286	0.253	0.032	0.048	0.034
RS854	0.288	0.231	0.201	0.220	0.349	0.274	0.031	0.068	0.049
Mean	**0.299**	**0.233**	**0.208**	**0.243**	**0.330**	**0.286**	**0.029**	**0.059**	**0.042**
Total mean	0.286	0.244	0.216	0.219	0.340	0.293	0.026	0.068	0.049
CD at 5%	0.012	0.026	0.044	0.016	0.034	0.059	0.001	0.007	0.012

(g/g/day) respectively, under very late planting 0.216, 0.293 and 0.049 (g/g/day) respectively. Under normal planting irrigated genotypes showed higher RGR values compared to rainfed genotypes at all the growth stages, while under late and very late plantings rainfed genotypes showed higher RGR values than irrigated genotypes (Table 10). The mean RGR values at the three growth stages were 0.233, 0.330 and 0.059 (g/g/day) and 0.208, 0.286 and 0.042 (g/g/day) under late and very late plantings respectively, while the mean values in rainfed genotypes were 0.252, 0.447 and 0.074 (g/g/day) and 0.222, 0.299 and 0.055 (g/g/day) respectively under late and very late plantings.

4.1.2.4 Net assimilation Rate

Net assimilation rate (NAR) under all plantings showed highest values at anthesis stages. The mean values under normal planting at vegetative, anthesis and at 15 days after anthesis were 0.26, 0.31 and 0.08 (mg/dm^2/day) respectively, under late planting 0.18, 0.59 and 0.15 (mg/dm^2/day) respectively and under very late planting 0.14, 0.45 and 0.27 (mg/dm^2/day) respectively (Table 11). The NAR mean values of irrigated genotypes under normal planting were higher than the rainfed genotypes, while under late and very late planting the rainfed genotypes showed higher NAR values than irrigated genotypes. The mean values of NAR at normal planting in irrigated genotypes were 0.296, 0.326 and 0.09 (mg/dm^2/day) respectively, under late planting 0.176, 0.574 and 0.45 (mg/dm^2/day) respectively, and under very late planting 0.131, 0.394 and 0.249 (mg/dm^2/day) respectively, while in the rainfed genotypes the values of NAR under normal planting were 0.239, 0.307 and 0.068 (mg/dm^2/day) respectively, under late planting 0.187, 0.603 and

Table 11. Net assimilation rate (mg/dm²/day) in twenty (irrigated and rainfed) wheat genotypes at different stages of growth (vegetative stage, anthesis and 15 days after anthesis) at all planting date (normal, late and very late)

Genotypes	Vegetative stage			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	0.24	0.19	0.16	0.33	0.65	0.59	0.08	0.19	0.37
DL-153-2	0.22	0.16	0.14	0.37	0.63	0.56	0.08	0.16	0.24
HD2781	0.19	0.13	0.10	0.33	0.58	0.48	0.04	0.16	0.23
HD2865	0.27	0.20	0.10	0.35	0.54	0.46	0.05	0.16	0.23
HD2866	0.23	0.17	0.13	0.30	0.46	0.35	0.07	0.17	0.30
HD2868	0.20	0.13	0.09	0.33	0.53	0.37	0.08	0.14	0.22
HDR-77	0.23	0.21	0.18	0.25	0.78	0.61	0.02	0.18	0.36
HI1500	0.22	0.18	0.16	0.17	0.77	0.61	0.17	0.11	0.33
RS887	0.28	0.23	0.19	0.32	0.48	0.36	0.04	0.15	0.30
RS888	0.28	0.20	0.16	0.23	0.53	0.45	0.06	0.14	0.31
K8027	0.28	0.24	0.19	0.30	0.63	0.45	0.06	0.14	0.34
PBW175	0.23	0.20	0.15	0.40	0.65	0.52	0.06	0.14	0.21
Mean	**0.24**	**0.19**	**0.15**	**0.31**	**0.60**	**0.48**	**0.07**	**0.15**	**0.29**
Irrigated									
HD2329	0.30	0.14	0.11	0.31	0.60	0.33	0.11	0.16	0.27
HD2815	0.26	0.20	0.16	0.33	0.68	0.53	0.08	0.17	0.31
HD2733	0.29	0.18	0.06	0.31	0.47	0.37	0.06	0.13	0.21
PBW343	0.36	0.17	0.16	0.35	0.59	0.39	0.12	0.17	0.23
WR544	0.35	0.22	0.16	0.31	0.67	0.48	0.10	0.18	0.32
RS827	0.28	0.18	0.14	0.32	0.53	0.35	0.08	0.14	0.19
RS873	0.27	0.15	0.13	0.31	0.54	0.38	0.09	0.12	0.19
RS854	0.26	0.17	0.13	0.37	0.51	0.32	0.08	0.13	0.27
Mean	**0.30**	**0.18**	**0.13**	**0.33**	**0.57**	**0.39**	**0.09**	**0.15**	**0.25**
Total mean	0.26	0.18	0.14	0.31	0.59	0.45	0.08	0.15	0.27
CD at 5%	0.003	0.031	0.055	0.022	0.049	0.085	0.006	0.025	0.044

0.153 (mg/dm^2/day) respectively, under very late planting 0.146, 0.484 and 0.287 (mg/dm^2/day) respectively.

4.1.2.5 Plant Height

Plant height under all plantings showed highest values at 15 days after anthesis. Significant reduction in plant height was recorded under late and very late planting compared to normal. There were 23.6, 26.5, 25.2 and 23.5% reductions under late planting compared to normal at 40, 60 DAS, anthesis and 15 days after anthesis respectively, while under very late planting the per cent reduction was 45.7, 59.5, 57.8 and 42.8% respectively. More reductions were recorded in irrigated genotypes than in rainfed genotypes under late and very late plantings compared to normal (Table 12). The per cent reduction in irrigated genotypes was 29.5, 30.8, 29.2 and 28% and 47.6, 65.5, 64.5 and 48% under late and very late plantings respectively. In the rainfed genotypes the per cent reduction was 19.3, 23.5, 22.3 and 20.2% and 44.3, 55.2, 53 and 38.9% under late and very late plantings respectively.

4.1.3 Yield and Yield Attributes

Yield and yield components were significantly affected by heat stress imposed by delayed planting.

4.1.3.1 Total Biomass

Under normal planting, irrigated genotypes showed higher biomass than rainfed ones. Significant reduction in total biomass was recorded under late and very late planting compared to normal. However, the per cent reduction in irrigated genotypes was higher than rainfed genotypes. Mean values of total biomass in irrigated genotypes under normal, late and very late

Table 12. Plant height (cm) in twenty (irrigated and rainfed) wheat genotypes at different stages of growth (40 days old seedling, 60 days old seedling, anthesis and 15 days after anthesis) at all planting date (normal, late and very late)

Genotypes	40 days old seedling			60 days old seedling			Anthesis stage			15 days after anthesis		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed												
C306	24.3	22.3	16.3	38.8	24.4	18.8	44.7	36.0	24.0	62.1	44.7	39.3
DL-153-2	23.2	20.2	15.7	28.8	25.7	17.4	38.7	31.6	19.3	64.9	44.7	36.0
HD2781	26.6	21.2	13.2	31.1	24.3	16.3	45.0	31.3	18.3	60.6	45.4	38.3
HD2865	22.4	18.4	13.2	32.3	26.3	18.8	51.0	38.7	20.3	58.0	44.7	34.0
HD2866	27.2	19.4	10.5	31.2	24.0	11.9	52.7	38.8	26.0	68.7	42.7	36.7
HD2868	28.3	18.6	12.3	36.2	23.3	14.6	42.8	34.0	16.7	64.7	57.7	40.0
HDR-77	24.0	21.7	17.2	33.3	26.7	19.2	39.9	33.6	27.0	62.0	53.7	46.0
HI1500	25.6	22.5	16.5	30.4	23.0	17.6	41.8	35.4	22.3	59.3	42.7	37.3
RS887	26.2	21.3	10.3	38.4	34.0	15.6	50.9	35.9	18.0	79.1	70.3	43.6
RS888	30.6	22.9	17.7	35.8	29.3	19.8	43.4	32.7	18.0	76.5	64.7	50.0
K8027	24.4	19.9	15.2	37.3	33.8	17.6	47.5	37.7	27.2	76.9	66.3	50.3
PBW175	26.2	20.9	14.2	29.8	23.6	16.4	37.0	30.1	17.3	51.9	49.0	28.0
Mean	**25.7**	**20.8**	**14.3**	**33.6**	**26.5**	**17.0**	**44.6**	**34.7**	**21.2**	**65.4**	**52.2**	**40.0**
Irrigated												
HD2329	18.5	14.0	34.9	26.3	16.6	44.1	31.8	18.3	66.3	51.2	21.0	18.5
HD2815	20.6	18.7	32.6	27.3	20.1	45.9	38.8	22.5	75.2	43.7	37.7	20.6
HD2733	17.1	11.2	32.3	19.0	13.9	43.4	30.8	15.0	70.9	32.0	39.3	17.1
PBW343	17.5	13.5	38.1	22.3	15.5	55.1	30.3	19.0	86.2	66.7	37.3	17.5
WR544	20.6	14.7	36.4	27.6	17.1	46.2	37.3	19.3	85.3	71.3	46.3	20.6
RS827	18.9	11.5	34.2	24.3	14.3	51.0	37.0	20.3	82.3	55.7	42.3	18.9
RS873	19.9	14.5	34.1	21.6	16.3	42.5	36.7	18.7	52.3	47.7	36.6	19.9
RS854	18.7	14.7	30.5	23.3	17.3	40.3	18.0	20.6	52.4	43.0	36.6	18.7
Mean	**19.0**	**14.1**	**34.1**	**24.0**	**16.4**	**46.1**	**32.6**	**19.2**	**71.4**	**51.4**	**37.1**	**19.0**
Total mean	20.2	14.2	33.8	25.5	16.7	45.2	33.8	20.4	67.8	51.9	38.8	20.2
CD at 5%	0.45	1.15	1.99	0.87	2.25	2.89	0.94	1.41	2.18	1.67	2.31	3.46

planting were 727.1, 529.6 and 214 g respectively, while rainfed genotypes showed 700.8, 546.1 and 229.4 g respectively (Table 13). Per cent reduction in total biomass in irrigated genotypes was 27.2 and 70.6% under late and very late plantings respectively, while rainfed genotypes the reduction was 22.1 and 67.3% respectively.

4.1.3.2 Grain Yield

Grain yield also reduced significantly under late and very late planting compared to normal and the influence of heat stress was more significant and clearly seen in irrigated genotypes. The mean values of grain yield under normal, late and very late planting in irrigated genotypes were 227.5, 174.9 and 65.5 g respectively, while the values were 216.2, 181.4 and 72.2 g respectively in rainfed genotypes (Table 13). The irrigated genotypes showed 23.1 and 71.2% reduction in grain yield under late and very late planting respectively, while rainfed genotypes showed 16.1 and 66.6% reduction respectively.

4.1.3.3 Harvest Index

In general harvest index increased under late and very late planting compared to normal, however among the genotypes HD 2781 and HD 2868 (rainfed) and HD 2329, PBW 343 (irrigated) showed reductions in harvest index under very late planting compared to normal. Irrigated genotypes showed higher harvest index compared to rainfed genotypes under normal planting and lower harvest index under late and very late planting. However, the difference was not significant under late and very late planting (Table 13).

Table 13. Plant biomass (g), grain yield (g) per two meter and harvest index (%) under normal late and very late planting

Genotypes	Total Biomass			Grain Yield			Harvest Index		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	663	533	245	208	186	80	31.4	34.9	32.5
DL-153-2	633	543	213	193	170	65	30.5	31.3	30.6
HD2781	691	608	212	195	183	63	28.3	30.1	29.8
HD2865	692	622	285	203	195	87	29.4	31.4	30.6
HD2866	782	587	252	252	198	78	32.2	33.8	31.1
HD2868	732	552	245	237	188	79	32.4	34.1	32.4
HDR-77	730	502	212	234	177	69	32.0	35.2	32.6
HI1500	720	540	237	220	170	76	30.6	31.5	32.0
RS887	663	538	209	218	192	68	32.9	35.6	32.6
RS888	690	502	199	231	171	67	33.5	34.1	33.7
K8027	723	549	239	208	177	71	28.8	32.2	29.6
PBW175	690	478	205	193	170	63	28.0	35.5	30.8
Mean	**701**	**546**	**229**	**216**	**181**	**72**	**30.8**	**33.2**	**31.5**
Irrigated									
HD2329	793	473	227	257	157	54	32.4	33.1	23.6
HD2815	697	527	205	211	188	66	30.2	35.6	32.1
HD2733	750	450	211	230	140	67	30.7	31.2	31.6
PBW343	690	510	188	210	168	55	30.5	33.0	29.3
WR544	767	610	240	265	183	84	34.6	30.0	34.9
RS827	743	607	215	212	195	69	28.5	32.1	31.9
RS873	680	513	208	227	178	62	33.3	34.7	29.6
RS854	697	547	219	209	190	69	30.1	34.8	31.7
Mean	**727**	**530**	**214**	**228**	**175**	**66**	**31.3**	**33.0**	**30.6**
Total mean	712	539	223	221	178	65	31.0	33.1	29.0
CD at 5%	9.17	10.11	12.82	4.76	8.36	10.41	0.79	1.85	3.21

4.1.3.4 Grain Number and Grain Weight per Ear

Grain number and grain weight per ear was significantly reduced under late and very late planting compared to normal. No significant difference in grain number between irrigated and rainfed genotypes under normal planting was observed but there was significant difference under late and very late plantings. Irrigated genotypes showed 44.6, 37.8 and 34.5 grain per ear under normal, late and very late planting respectively, while rainfed genotypes showed 44.3, 40.2 and 37 respectively. Per cent reduction in grain number per ear in irrigated genotypes was 15.2 and 22.3% under late and very late plantings respectively, while in rainfed genotypes reduction was 9.3 and 16.6% respectively. Grain weight per ear was also adversely affected by late planting and there was 19.5 and 32.4% reduction in grain weight per ear under late and very late planting respectively. Irrigated genotypes showed more reduction in grain weight per ear compared to rainfed genotypes under late and very late plantings. The per cent reduction in irrigated genotypes under late and very late planting compared to normal was 19.5 and 32.4% respectively, while the reduction in rainfed genotypes was 16.1 and 26.5% respectively (Table 14).

4.1.3.5 1000 Grain Weight

1000 grain weight was significantly reduced under late plantings compared to normal and irrigated genotypes showed more reduction in 1000 grain weight under late planting conditions than rainfed genotypes. The per cent reduction in irrigated genotypes under late and very late planting was 14 and 26% respectively, while rainfed genotypes showed 8.9 and 22.2% respectively (Table 14).

Table 14. Grain number and grain weight (g) per ear and 1000 grain weight (g) under normal late and very late planting

Genotypes	Grain number per ear			Grain weight per ear			1000-grain weight		
	Normal	Late	Very Late	Normal	Late	Very Late	Normal	Late	Very Late
Rainfed									
C306	41.3	41.0	39.3	1.17	1.00	0.98	37.1	33.6	29.3
DL-153-2	47.0	43.0	39.7	1.53	1.31	1.19	38.9	33.6	30.1
HD2781	46.7	40.3	34.3	1.19	1.07	0.70	35.2	33.0	28.9
HD2865	41.7	39.0	38.7	1.37	1.23	1.08	36.8	35.6	29.4
HD2866	45.0	40.3	38.7	1.26	1.08	0.93	31.0	30.3	26.9
HD2868	45.0	41.3	38.7	1.56	1.16	0.88	39.4	34.1	29.6
HDR-77	43.7	39.7	36.0	1.48	1.11	1.10	36.7	33.7	26.2
HI1500	46.0	40.0	33.3	1.35	1.05	1.00	34.5	30.3	27.4
RS887	39.7	38.7	36.0	1.16	1.03	0.86	34.9	33.2	26.7
RS888	44.7	39.0	37.0	1.25	1.11	0.98	38.1	34.3	30.5
K8027	47.0	41.3	34.7	1.14	1.06	1.03	35.2	32.8	26.2
PBW175	44.3	38.7	37.3	1.55	1.22	1.03	36.9	31.7	27.0
Mean	**44.3**	**40.2**	**37.0**	**1.33**	**1.12**	**0.98**	**36.2**	**33.0**	**28.2**
Irrigated									
HD2329	46.7	35.3	33.3	1.55	1.04	0.85	39.8	30.0	24.1
HD2815	46.0	40.7	37.0	1.35	1.19	0.99	36.9	27.1	24.8
HD2733	46.7	34.7	33.0	1.52	1.08	0.75	41.3	32.8	26.4
PBW343	37.0	36.7	31.7	1.41	1.09	0.58	37.6	31.2	23.8
WR544	45.3	40.3	38.0	1.39	1.10	0.96	39.7	36.6	32.6
RS827	45.0	39.3	33.7	1.35	1.03	0.79	36.8	25.1	24.9
RS873	46.0	38.3	34.3	1.46	1.07	0.85	37.8	30.4	24.3
RS854	44.0	37.0	36.0	1.32	1.02	1.01	38.6	30.3	28.7
Mean	**44.6**	**37.8**	**34.6**	**1.42**	**1.08**	**0.85**	**38.6**	**30.4**	**26.2**
Total mean	44.4	39.2	36.0	1.37	1.10	0.93	37.2	32.0	27.4
CD at 5%	0.492	2.052	3.555	0.272	0.069	0.120	0.309	1.335	2.312

4.1.3.6 Heat Use Efficiency (Biological Yield)

The mean values for heat unit efficiency under normal, late and very late planting were 1.140, 0.978, and 0.358 (g plant^{-1} ^{0} day^{-1}) respectively. However, the mean values for heat unit efficiency in rainfed genotypes were 1.089, 0.972 and 0.367 (g plant^{-1} ^{0} day^{-1}) under normal, late and very late planting respectively, while it was 1.215, 0.987 and 0.345(g plant^{-1} ^{0} day^{-1}) respectively in the irrigated genotypes (Table 15).

4.1.3.7 Heat Use Efficiency (Grain Yield)

There was significant decrease in the heat unit efficiency values under late and very late planting compared to normal planting. However, rainfed genotypes showed the following values under normal, late and very late planting: 0.345, 0.325 and 0.114 (g plant^{-1} ^{0} day^{-1}) units respectively, while irrigated genotypes showed 0.375, 0.326 and 0.106 (g plant^{-1} ^{0} day^{-1}) units respectively (Table 16).

4.1.3.8 Yield Stability Index

The yield were more stable under late and very late planting in rainfed genotype compared to irrigated genotype, the mean value for yield stability in rainfed genotypes was 80.61 and 37.72 % under late and very late planting respectively, while it was 74.08 and 28.94 % respectively in irrigated genotypes (Table 17).

4.1.3.9 Heat Susceptibility Index (Grain Yield)

The value of heat susceptibility was 0.732 and 0.959 g plant^{-1} in rainfed genotypes under late and very late planting respectively, while it was 0.934 and 1.23 g plant^{-1} respectively in irrigated genotypes (Table 18).

Table 15. Heat use efficiency (g plant^{-1} ^{0} day^{-1}) of biological yield in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting

Genotypes	Normal	Late	Very Late
Rainfed			
C306	0.97	0.95	0.43
DL-153-2	1.03	0.94	0.31
HD2781	1.09	1.06	0.31
HD2865	1.11	1.12	0.47
HD2866	1.27	1.06	0.40
HD2868	1.19	1.04	0.40
HDR-77	1.06	0.87	0.40
HI1500	1.04	0.96	0.38
RS887	1.08	0.96	0.31
RS888	1.09	0.91	0.32
K8027	1.06	0.94	0.38
PBW175	1.10	0.86	0.31
Mean	1.09	0.97	0.37
Irrigated			
HD2329	1.33	0.86	0.38
HD2815	1.15	1.03	0.34
HD2733	1.06	0.77	0.31
PBW343	1.38	0.86	0.31
WR544	1.32	1.24	0.42
RS827	1.24	1.16	0.32
RS873	1.10	1.00	0.33
RS854	1.13	0.99	0.35
Mean	1.22	0.99	0.35
Total mean	1.33	0.86	0.38
CD at 5%	0.254	0.357	0.384

Table 16. Heat use efficiency (g plant^{-1} ^{0} day^{-1}) of grain yield in twenty (irrigated and rainfed) wheat genotypes under normal, late and very late planting

Genotypes	Normal	Late	Very Late
Rainfed			
C306	0.306	0.342	0.122
DL-153-2	0.313	0.296	0.105
HD2781	0.308	0.318	0.093
HD2865	0.325	0.352	0.143
HD2866	0.408	0.358	0.125
HD2868	0.384	0.354	0.130
HDR-77	0.383	0.307	0.114
HI1500	0.347	0.319	0.121
RS887	0.354	0.340	0.100
RS888	0.364	0.309	0.107
K8027	0.336	0.302	0.111
PBW175	0.309	0.307	0.095
Mean	0.345	0.325	0.114
Irrigated			
HD2329	0.430	0.283	0.090
HD2815	0.347	0.366	0.108
HD2733	0.326	0.240	0.098
PBW343	0.376	0.282	0.090
WR544	0.457	0.371	0.147
RS827	0.354	0.373	0.103
RS873	0.367	0.347	0.099
RS854	0.339	0.343	0.111
Mean	0.375	0.326	0.106
Total mean	0.357	0.325	0.111
CD at 5%	2.31	3.05	2.09

Table 17. Yield stability index (%) under late and very late planting in twenty (Irrigated and rainfed) wheat genotypes

Genotypes	Late	Very Late
Rainfed		
C306	89.28	46.57
DL-153-2	87.93	43.47
HD2781	78.69	32.42
HD2865	76.87	42.95
HD2866	78.81	31.12
HD2868	79.58	33.52
HDR-77	84.57	45.27
HI1500	83.59	44.39
RS887	73.59	31.15
RS888	73.92	28.96
K8027	84.80	40.28
PBW175	75.68	32.59
Mean	**80.61**	**37.72**
Irrigated		
HD2329	61.04	20.91
HD2815	85.42	31.17
HD2733	61.01	28.98
PBW343	73.26	26.15
WR544	81.30	31.57
RS827	69.48	32.44
RS873	78.53	27.20
RS854	82.56	33.12
Mean	**74.08**	**28.94**
Total mean	77.94	34.13
CD at 5%	4.37	7.84

Table 18. Heat susceptibility index of grain yield (g plant^{-1}) in twenty (irrigated and rainfed) wheat genotypes under late and very late planting

Genotypes	Late	Very Late
Rainfed		
C306	0.538	0.826
DL-153-2	0.561	0.822
HD2781	0.307	0.848
HD2865	0.215	0.746
HD2866	1.252	1.111
HD2868	1.137	1.009
HDR-77	0.668	0.891
HI1500	0.748	0.928
RS887	0.638	0.964
RS888	1.413	1.642
K8027	0.753	0.884
PBW175	0.560	0.837
Mean	**0.732**	**0.959**
Irrigated		
HD2329	1.654	2.025
HD2815	0.553	0.931
HD2733	1.235	1.684
PBW343	1.337	0.996
WR544	0.674	0.842
RS827	0.407	0.918
RS873	1.144	1.546
RS854	0.468	0.899
Mean	**0.934**	**1.230**
Total mean	**0.815**	**1.070**
CD at 5%	0.242	0.349

4.2 EXPERIMENT-2

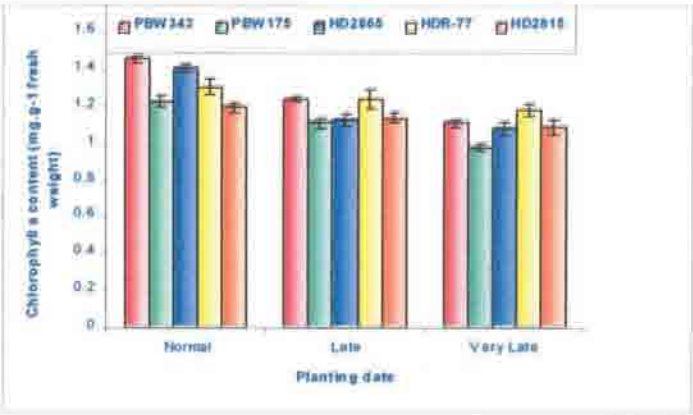
There was significant increase in temperature under late and very late planting compared to normal at all stages of growth (Fig. 2). This showed the significant effect of heat stress imposed to plants under late planting conditions.

4.2.1 Physiological and Biochemical Parameters

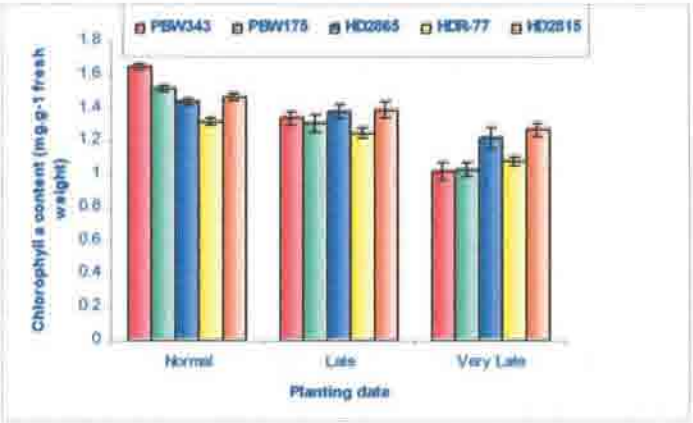
4.2.1.1 Chlorophyll Content

Significant reduction in chlorophyll content under late and very late planting compared to normal was recorded in all genotypes at all stages of growth and highest reduction was observed at anthesis stage. Under normal planting condition highest chlorophyll-a content was recorded at vegetative and anthesis stages in PBW 343 while at 15 days after anthesis in HD 2865. Under late planting highest chlorophyll-a content at anthesis stage and 15 days after anthesis was recorded in HD 2815, while at vegetative stage in HDR-77. Highest chlorophyll-a content under very late planting was recorded in HDR-77 at vegetative stage and at 15 days after anthesis, while at anthesis stage HD 2815 showed highest chlorophyll-a content (Fig. 4a, b and c). Highest chlorophyll-b content at vegetative stage under normal planting was recorded in HDR-77, at anthesis stage in HD 2865, and at 15 days after anthesis in PBW 343. Under late planting highest chlorophyll-b content was recorded in HDR-77 at vegetative and anthesis stage and in HD 2815 at 15 days after anthesis and HDR-77 showed highest chlorophyll-b content under very late planting at all stages of growth (Fig. 5a, b and c). Chlorophyll-a:b ratio increased significantly under late and very late planting compared to normal planting at all stages of growth. PBW 343 showed highest a:b ratio

(a)



(b)



(c)

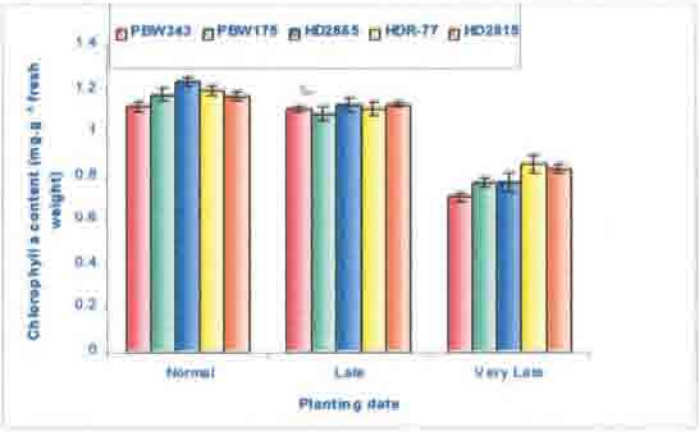
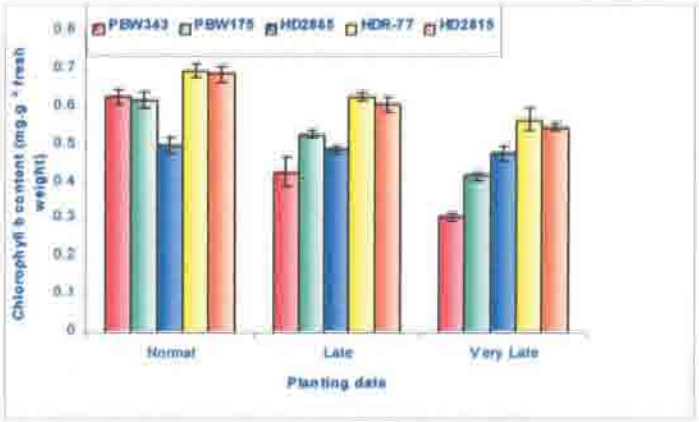
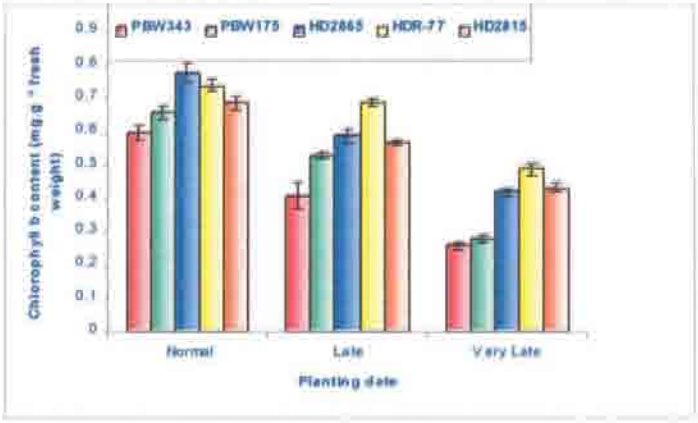


Fig. 4a, b and c. Chlorophyll a content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively.

(a)



(b)



(c)

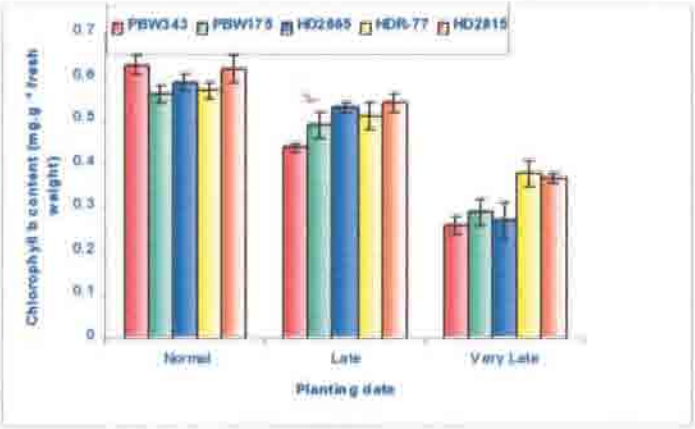
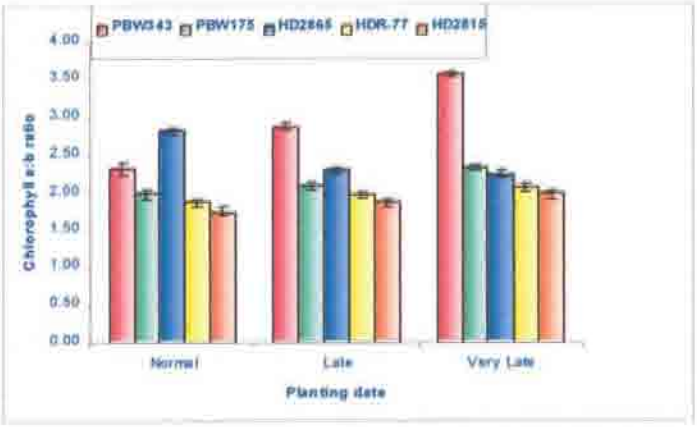
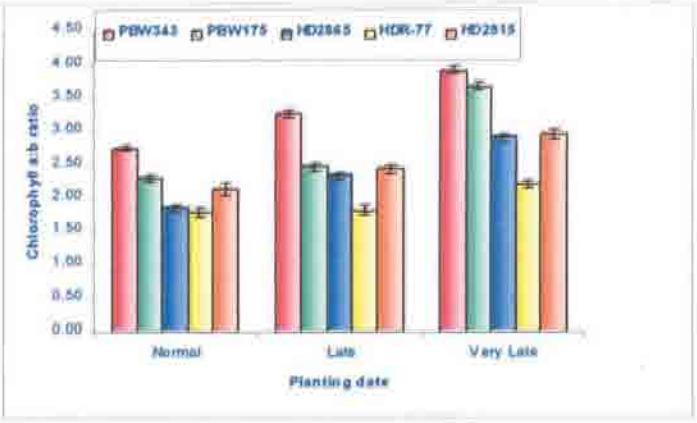


Fig. 5a, b and c. Chlorophyll b content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively

(a)



(b)



(c)

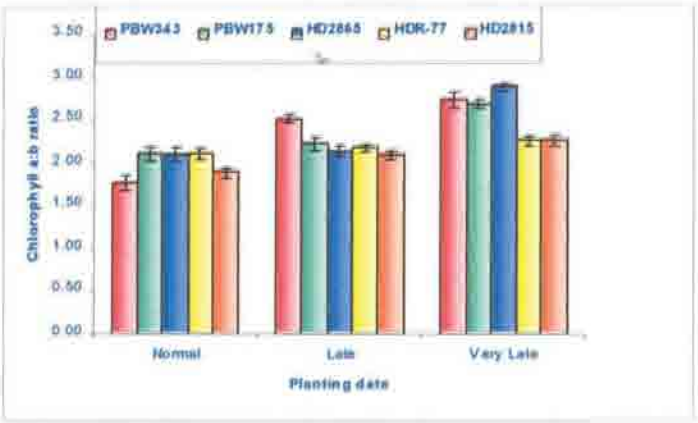
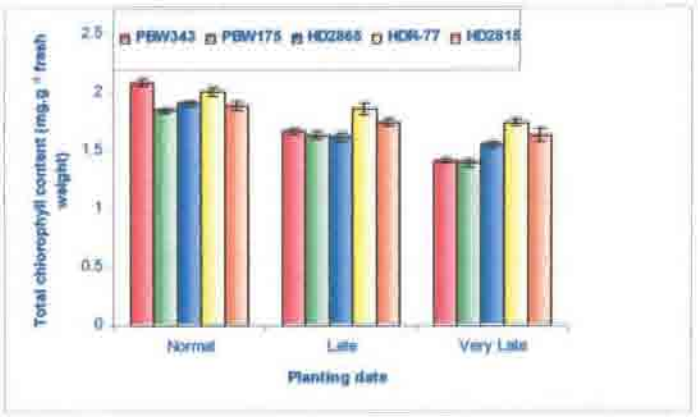
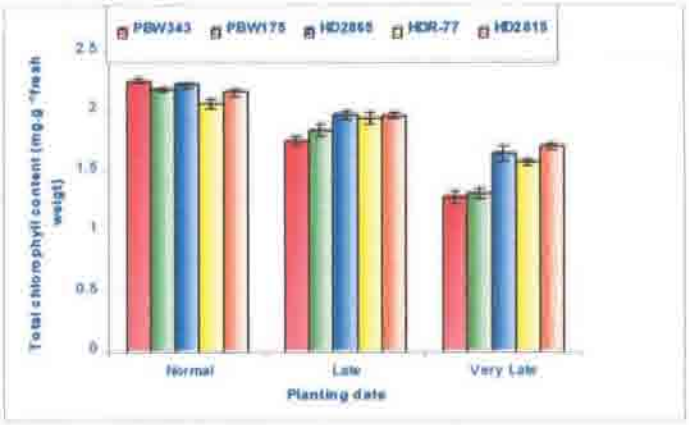


Fig. 6a, b and c. Chlorophyll a:b ratio in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively

(a)



(b)



(c)

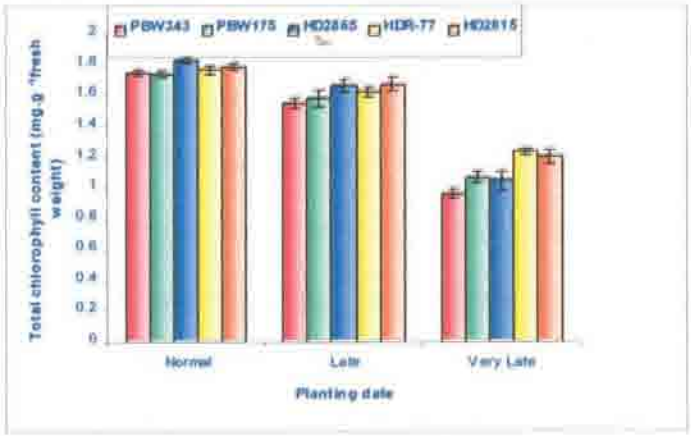


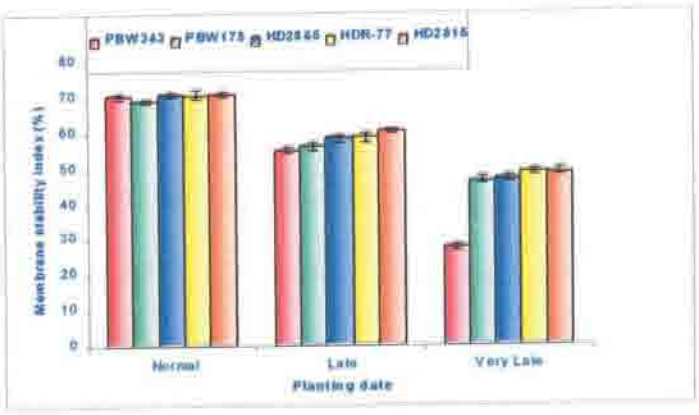
Fig 7a, b and c. Total chlorophyll content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively.

particularly under late planting conditions and highest a:b ratio recorded at anthesis stage (Fig. 6a, b and c). Highest total chlorophyll content under normal planting was recorded in PBW 343 at vegetative and anthesis stage and in HD 2865 at 15 days after anthesis. Under late planting highest value was recorded in HDR-77 at vegetative stage, in HD 2865 at anthesis stage and in HD 2815 at 15 days after anthesis. However, under very late planting highest value was recorded in HDR-77 at vegetative stage and at 15 days after anthesis, in HD 2815 at anthesis stage. Highest reduction in total chlorophyll content at vegetative stage, anthesis and 15 days after anthesis was recorded in PBW 343 i.e., 20, 22 and 11% respectively under late planting and 32, 43 and 44% respectively under very late planting and minimum reduction was recorded in HDR-77 and HD 2815 at all stages of growth under late planting conditions (Fig. 7a, b and c).

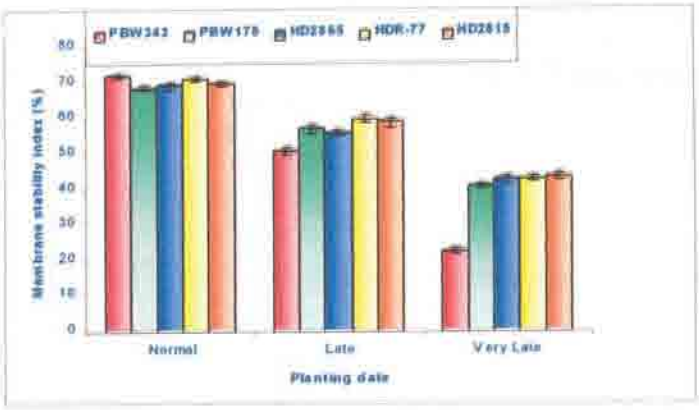
4.2.1.2 Membrane Stability Index

Highest membrane stability index value was recorded in HD 2815 at vegetative stage and at 15 days after anthesis under normal planting, while PBW 343 showed highest MSI at anthesis stage. Under late and very late planting highest MSI was recorded in HD 2815 and HDR-77 at all stages of growth. However, maximum reduction in MSI under late planting was recorded in PBW 343 i.e., 22, 29 and 22% at vegetative stage, anthesis and at 15 days after anthesis respectively, while under very late planting the per cent of reduction in PBW 343 was 61, 69 and 64% respectively and minimum reduction was recorded in HD 2815 and HDR-77 under late and very late planting at all stages of growth (Fig. 8a, b and c).

(a)



(b)



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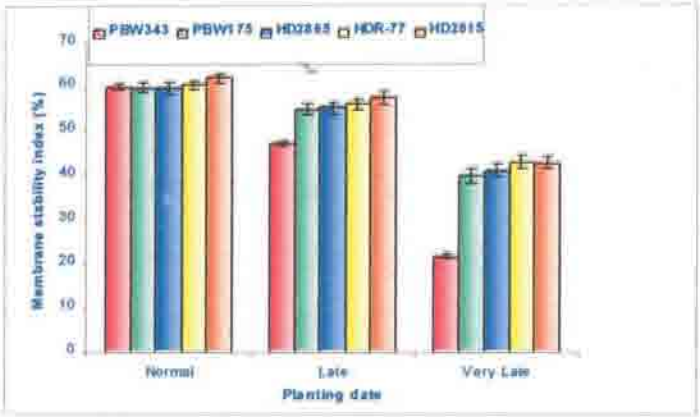


Fig. 8a, b and c. Membrane stability index (%) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively.

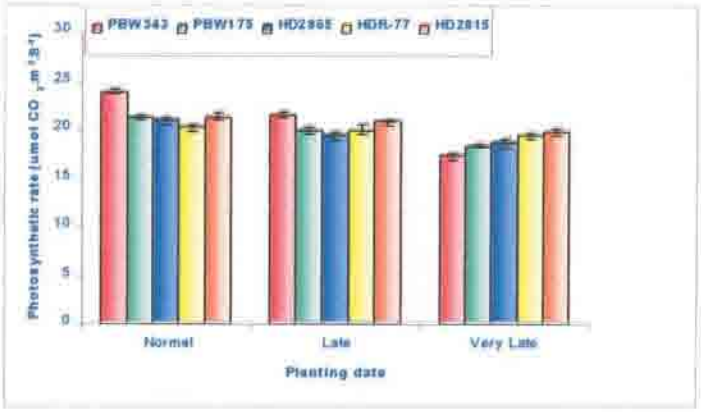
4.2.1.3 Photosynthetic Rate

Maximum photosynthetic rate under normal planting at all the growth stages was recorded in PBW 343, while under late planting condition highest Pn rate at vegetative and anthesis stage was recorded in PBW 343 and at 15 days after anthesis in HD 2865. Highest Pn rate value under very late planting at vegetative stage and at 15 days after anthesis was recorded in HD 2815, at anthesis in HD 2865. However, maximum reduction in photosynthetic rate under late planting compared to normal at vegetative stage and at 15 days after anthesis was recorded in PBW 343 i.e., 10, 12% respectively, at anthesis stage in HD 2865 i.e., 14%. Under very late planting maximum reduction in photosynthetic rate compared to normal at vegetative stage, anthesis and 15 days after anthesis was recorded in PBW 343 i.e., 28, 46 and 43% respectively and minimum reduction in photosynthetic rate under late planting condition compared to normal was recorded in HD 2815 and HDR-77 at all stages of growth (Fig. 9a, b and c).

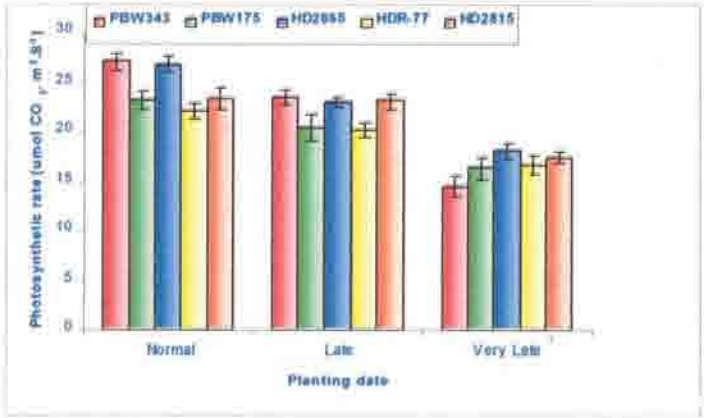
4.2.1.4 Leaf Water Transverse Relaxation Time (T_2)

A highest T_2 values were recorded at vegetative stage in all genotypes under all the plantings. Under normal planting highest T_2 value was recorded in PBW 343 at vegetative and anthesis stages and in HD 2865 at 15 days after anthesis. Under late planting highest T_2 value was recorded in PBW 175 at vegetative and anthesis stage and in HD 2815 at 15 days after anthesis. Under very late planting highest T_2 value was recorded in HDR-77 at vegetative stage and in HD 2815 at anthesis and at 15 days after anthesis. Maximum reduction in T_2 value at late planting was recorded in PBW 343 i.e., 21, 37 and 23% respectively and under very late planting PBW 343 showed

(a)



(b)



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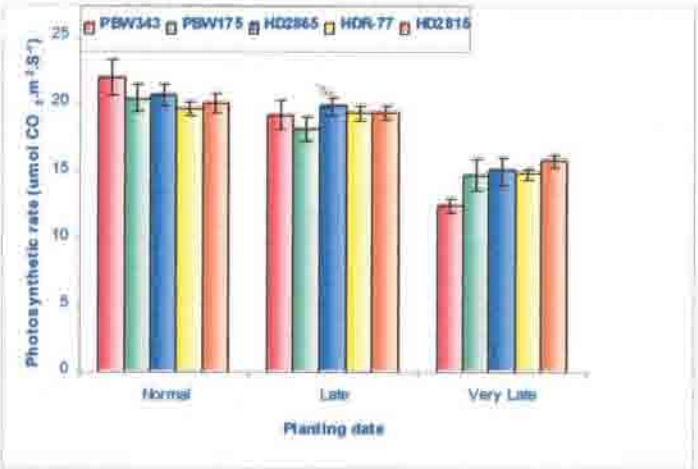
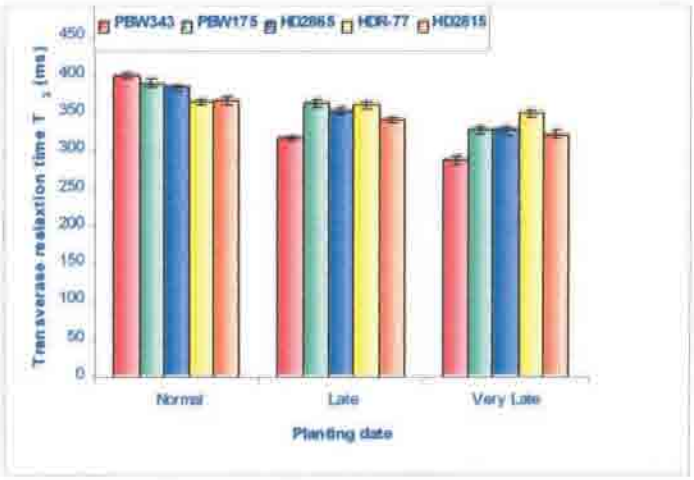
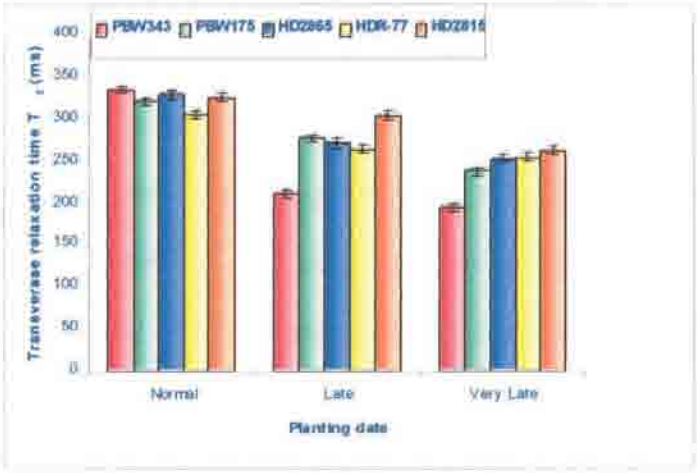


Fig. 9a, b and c. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively.

(a)



(b)



(c)

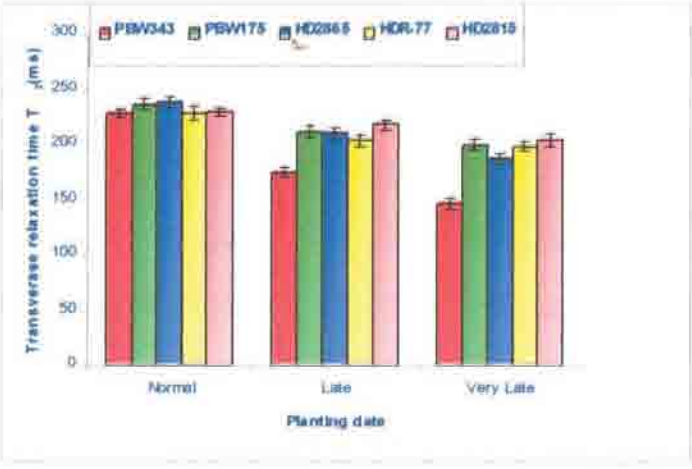


Fig. 10a, b and c. Transverse relaxation time T_2 (ms) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively.

also maximum reduction in T_2 value i.e., 29, 41 and 35% respectively. However comparatively HDR-77 and HD 2815 showed minimum reduction in T_2 value under late planting conditions at all stages of growth (Fig. 10a, b and c).

4.2.1.5 Water Potential

Highest leaf water potential values in all genotypes under all the three plantings were recoded at anthesis stage. However, at anthesis stage under normal planting highest value was recorded in HD 2865, at 7 days and 14 days after anthesis in PBW 343, while under late planting highest value for water potential at all stages was recorded in HD 2815. Under very late planting highest water potential value was recorded in HD 2815 at anthesis stage, in HDR-77 at 7 days after anthesis stage and in PBW 175 at 14 days after anthesis. However, maximum reduction in water potential at anthesis stage, 7 and 14 days after anthesis under late planting compared to normal was recorded in PBW 343 i.e., 20, 23 and 17% respectively, while under very late planting maximum reduction in water potential compared to normal was recorded in PBW 343 i.e., 40, 42 and 32% respectively. Minimum reduction in water potential at all stages of growth under late planting conditions compared to normal was recorded in HD 2815 and HDR-77 (Table 19a, b and c).

4.2.1.6 Superoxide Dismutase

There was significant increased in SOD activity at all stages of growth under late and very late planting compared to normal. However, under normal planting highest SOD activity at vegetative and anthesis stage was recorded in PBW 343 and at 15 days after anthesis in HD 2815, while under late and

Table 19a,b and c. Water potential (MPa) in five wheat genotypes under normal, late and very late planting, at anthesis stage, 7 and 14 days after anthesis respectively.

(a)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-1.88	-2.25	-2.63
PBW175	-1.97	-2.26	-2.47
HD2865	-1.8	-2.05	-2.23
HDR-77	-1.96	-2.16	-2.38
HD2815	-1.87	-2.03	-2.21
CD at 5%	0.054	0.068	0.098

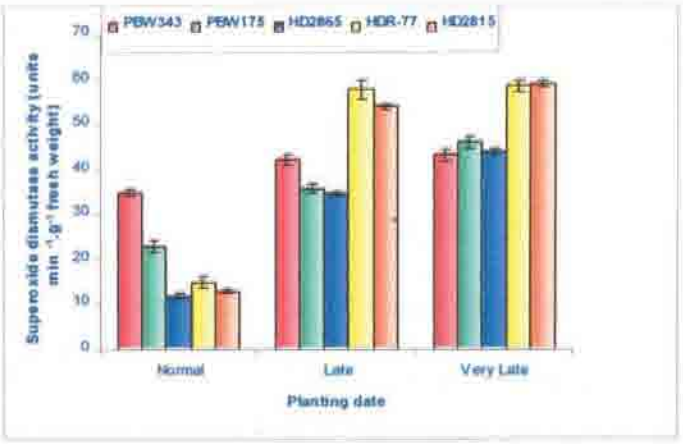
(b)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-1.75	-2.16	-2.48
PBW175	-1.81	-2.07	-2.26
HD2865	-1.93	-2.13	-2.34
HDR-77	-1.83	-2.06	-2.16
HD2815	-1.89	-2.01	-2.23
CD at 5%	0.023	0.064	0.078

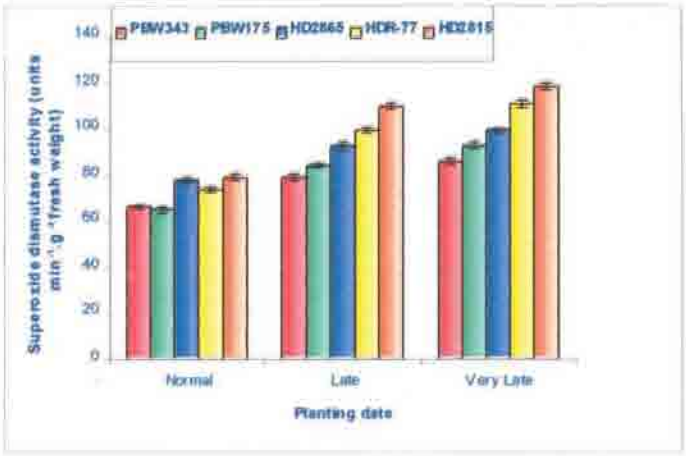
(c)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-2.18	-2.56	-2.87
PBW175	-2.1	-2.27	-2.57
HD2865	-2.09	-2.24	-2.47
HDR-77	-2.11	-2.18	-2.29
HD2815	-2.06	-2.16	-2.3
CD at 5%	0.036	0.068	0.084

a



b



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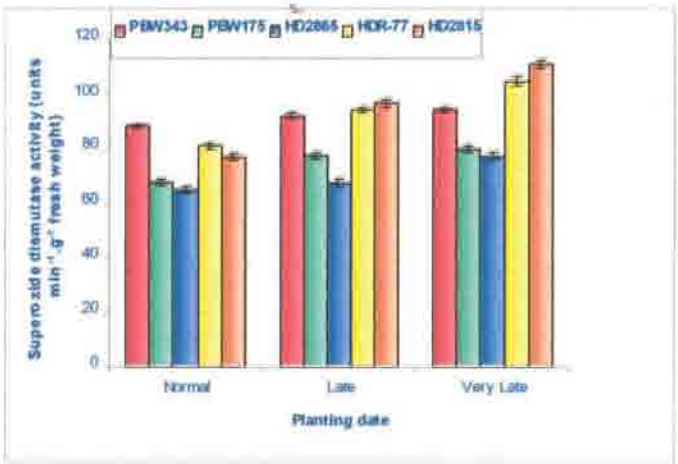


Fig. 11a, b and c. Superoxide dismutase activity (unit's min^{-1} g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively

very late planting highest SOD activity was recorded in HDR-77 and HD 2815 at all the growth stages (Fig. 11a, b and c).

4.2.1.7 Catalase

Catalase activity increased significantly under late and very late planting compared to normal. Under normal planting condition highest catalase activity was recorded in PBW 343 at vegetative stage, in HD 2865 at anthesis stage and in HDR-77 at 15 days after anthesis. Under late planting and very late plantings highest catalase activity was recorded in HD 2815 at vegetative stage, HDR-77 at anthesis stage and at 15 days after anthesis (Fig. 12a, b and c).

4.2.2 Yield and Yield attributes

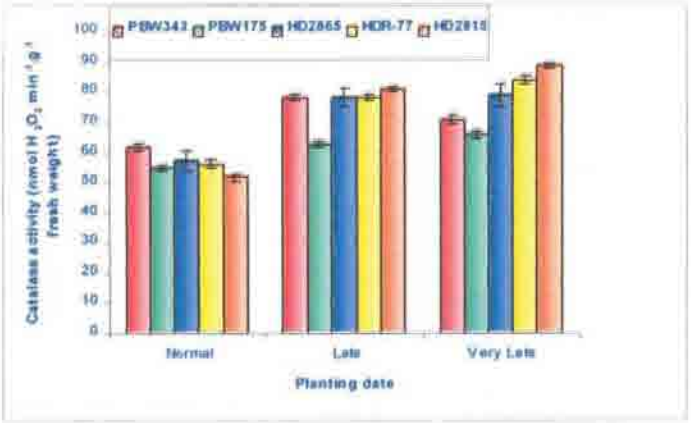
4.2.2.1 Total Biomass per Plant

Total biomass per plant decreased significantly under late and very late planting compared to normal. Under normal planting highest biomass per plant was recorded in PBW 343, under late planting highest value was recorded in HDR-77, while under very late planting in HD 2815. However, maximum reduction in total biomass was recorded in HD 2865 and PBW 343 under late planting compared to normal, while under very late planting maximum reduction was recorded in PBW 175, while minimum reduction under late and very late planting compared to normal was recorded in HDR-77 and HD 2815 (Fig. 13).

4.2.2.2 Grain Yield per Plant

Reduction in grain yield per plant was clearly seen under late and very late planting compared to normal. A highest grain yield value was recorded in PBW 175 at all the plantings. Maximum reduction in grain yield under late and

a



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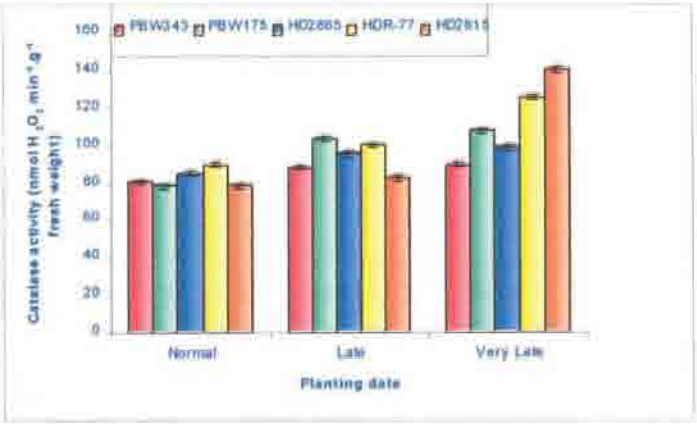

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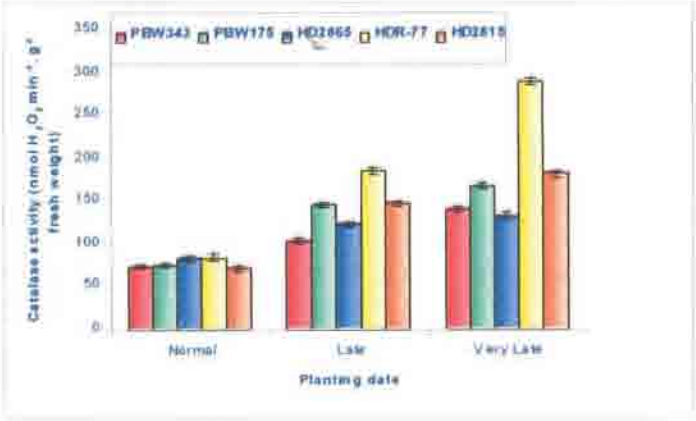


Fig. 12a, b and c. Catalase activity (nmol H_2O_2 min^{-1} g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting at vegetative, anthesis and 15 days after anthesis respectively

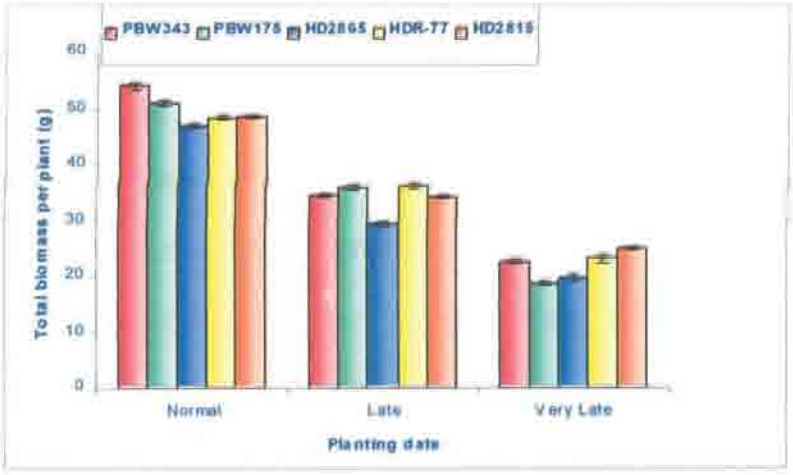


Fig. 13. Total biomass per plant (g) in five wheat genotypes under normal, late and very late planting

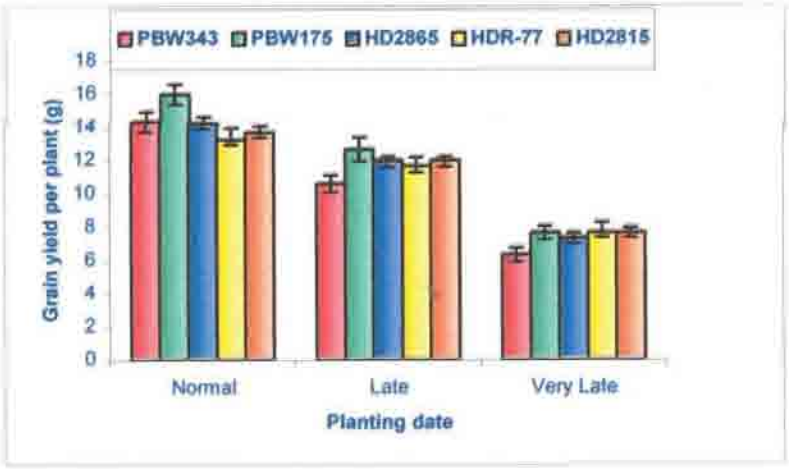


Fig. 14. Grain yield per plant (g) in five wheat genotypes under normal, late and very late planting

very late planting compared to normal was recorded in PBW 343 i.e., 26, 56%, while minimum reduction was recorded in HD 2815 and HDR-77 under late plantings (Fig. 14).

4.2.2.3 Grain Number and Grain Weight per Ear

Significant reduction in grain number and grain weight per ear was recorded under late and very late plantings compared to normal. Under normal and very late planting maximum number of grain per ear was recorded in HDR-77, while under late planting in HD 2815. Highest reduction in grain number per ear was recorded in PBW 343 under late and very late planting compared to normal i.e., 15, 25% respectively, while minimum reduction was recorded in HD 2815 under late planting conditions (Fig. 15). Highest grain weight per ear was recorded in PBW 175 under normal planting, HD 2815 under late and very late plantings and maximum reduction in grain weight per ear was recorded in PBW 343 under late and very late planting compared to normal i.e., 28, 41% respectively, while minimum reduction was recorded in HD 2865 under late planting and HD 2815 under very late planting (Fig. 16).

4.2.2.4 1000 Grain Weight

1000 grain weight was significantly reduced under late and very late planting compared to normal and highest value for 1000 grain weight was recorded in HD 2815 under normal, late and very late planting, maximum reduction in 1000 grain weight under late and very late planting compared to normal was recorded in PBW 343 i.e., 11, 16% respectively. Minimum reduction was observed in HD 2815 (Fig. 17).

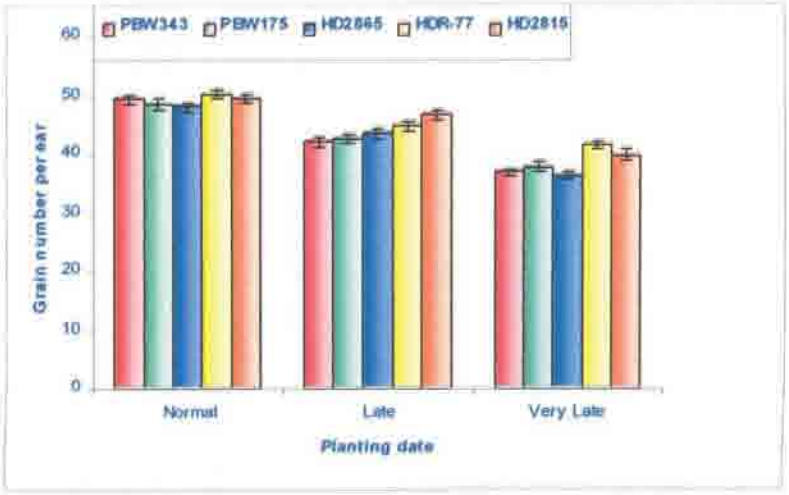


Fig. 15. Grain number per ear in five wheat genotypes under normal, late and very late planting

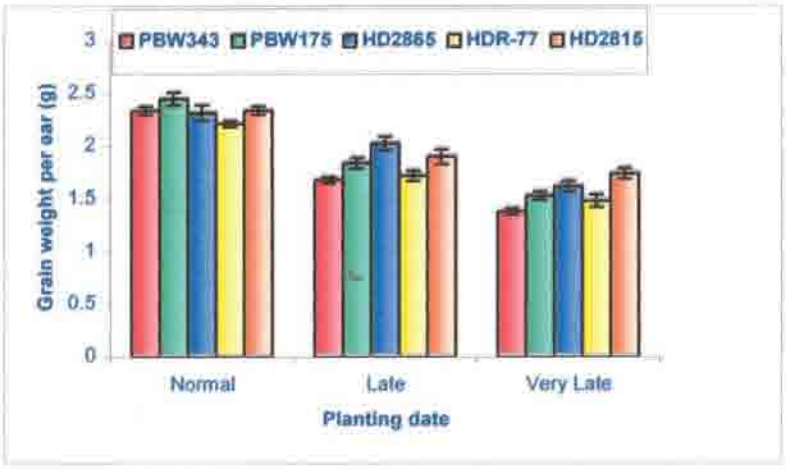


Fig 16. Grain weight per ear (g) in five wheat genotypes under normal, late and very late planting

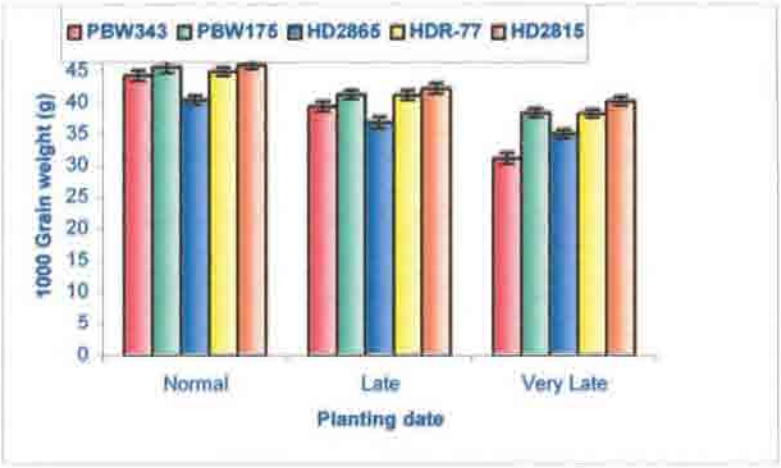


Fig 17. 1000 Grain weight (g) in five wheat genotypes under normal, late and very late planting

4.3 EXPERIMENT-3

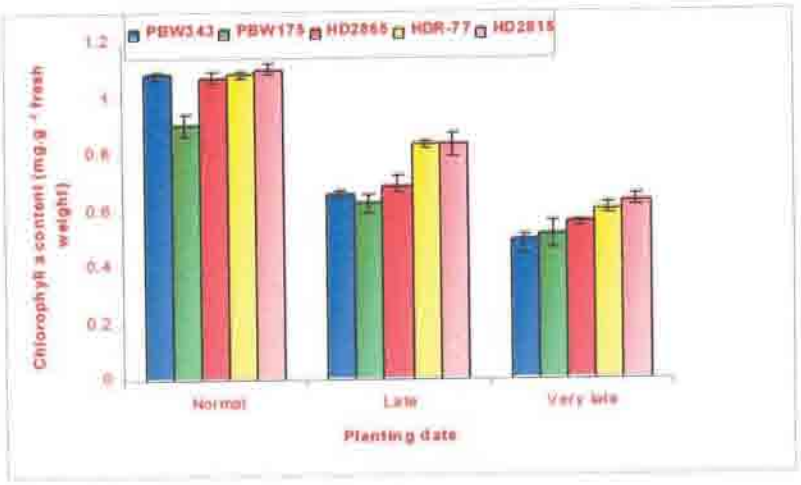
The significant increase in temperature under late and very late planting showed the effect of heat stress on plant grown late at all the growth stages (Fig. 3).

4.3.1 Physiological and Biochemical Parameters

4.3.1.1 Chlorophyll Content

Chlorophyll-a content was decreased significantly under late and very late planting compared to normal planting. Highest chlorophyll-a content under normal planting was recorded in HD 2815 at vegetative stage and at 15 days after anthesis, while highest value was recorded in PBW 343 at anthesis stage. Highest chlorophyll-a content under late and very late plantings at all stages of growth was recorded in HD 2815 (Fig. 18a, b and c). Chlorophyll-b content also significantly decreased under late planting conditions and highest chlorophyll-b content under normal planting was recorded in PBW 343 at vegetative and anthesis stage and in PBW 175 at 15 days after anthesis. Under late and very late plantings highest chlorophyll-b content was recorded in HD 2815 at all stages of growth (Fig. 19a, b and c). Chlorophyll a:b ratio increased significantly under late and very late planting compared to normal. Under normal planting highest chlorophyll a:b ratio was recorded in HD 2865 at vegetative stage and at 15 days after anthesis and in HDR-77 at anthesis stage, while under late planting highest value was recorded in HDR-77 at vegetative stage and at 15 days after anthesis and in PBW 175 at anthesis stage. Under very late planting highest ratio was recorded in PBW 343 at vegetative stage and at 15 days after anthesis and in HD 2865 at anthesis stage (Fig. 20a, b and c). Significant reduction in total chlorophyll content was

a



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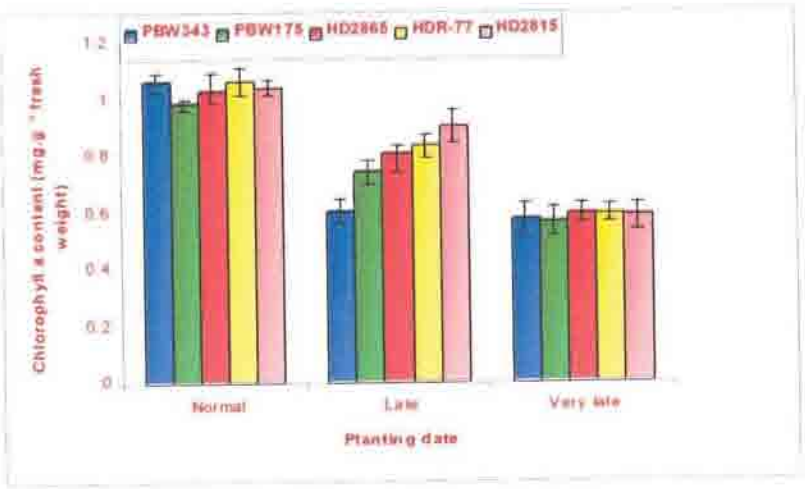

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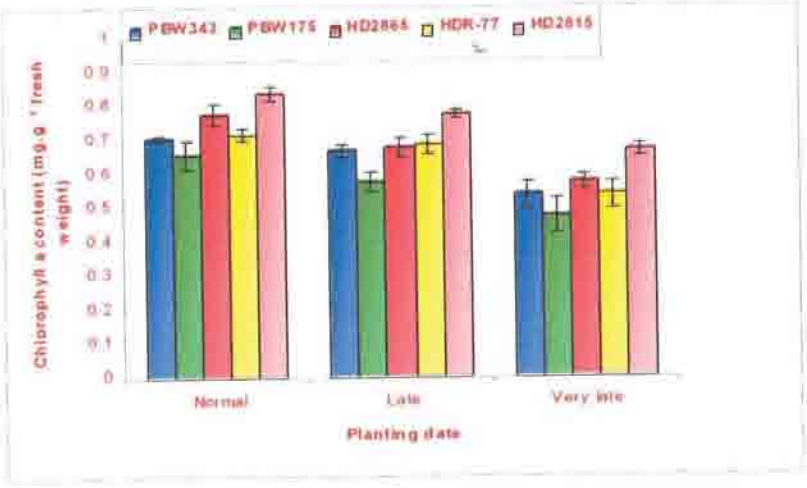
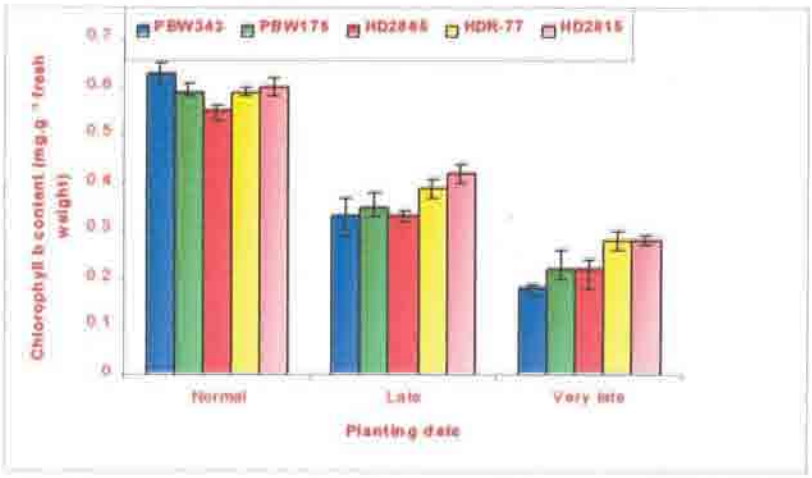


Fig. 18a, b and c. Chlorophyll a content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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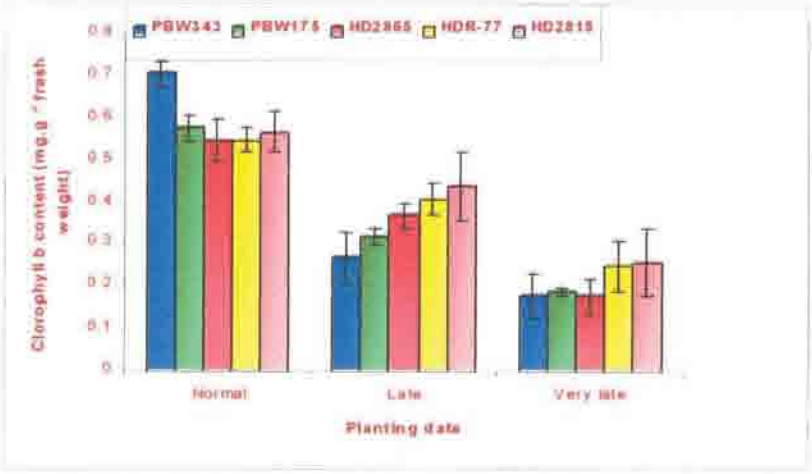

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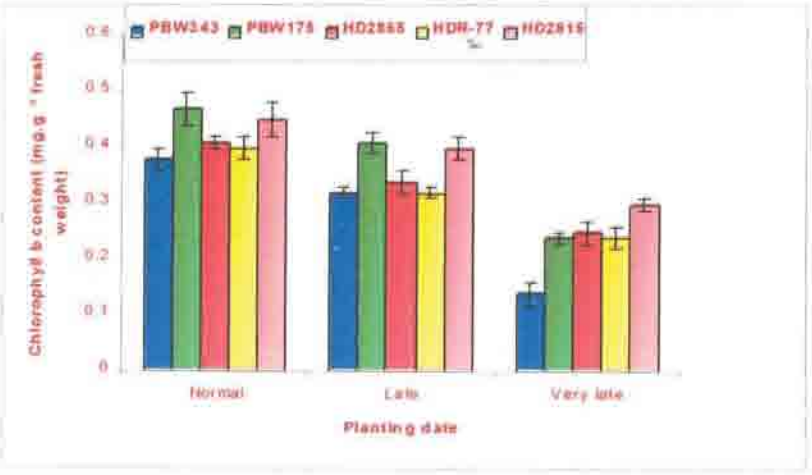
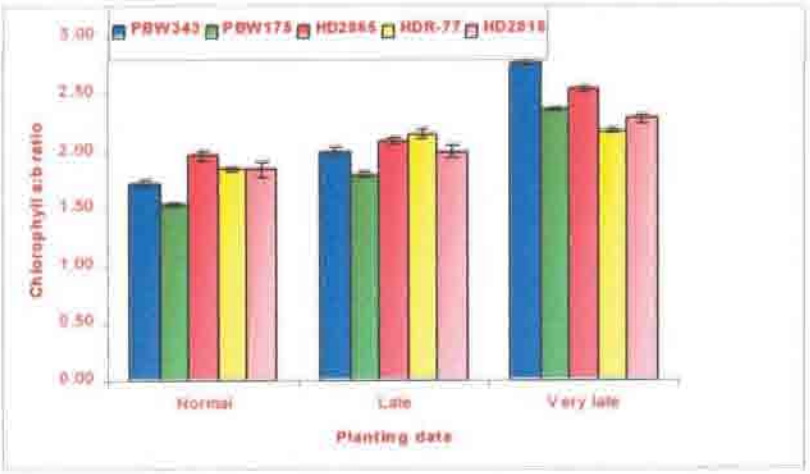


Fig. 19a, b and c. Chlorophyll b content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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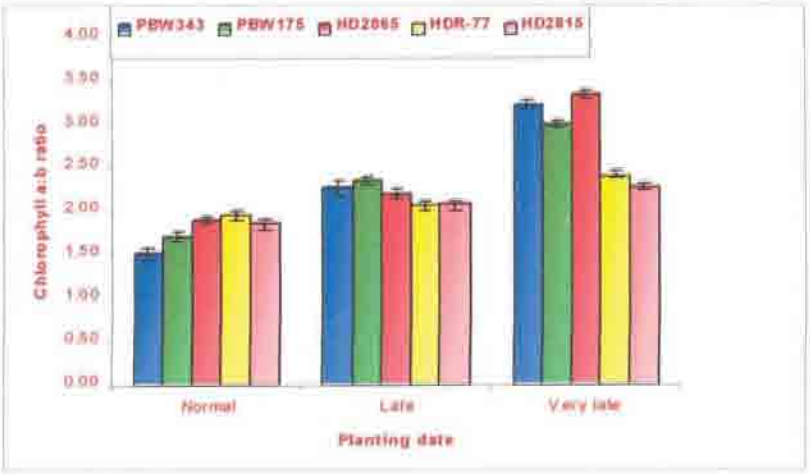

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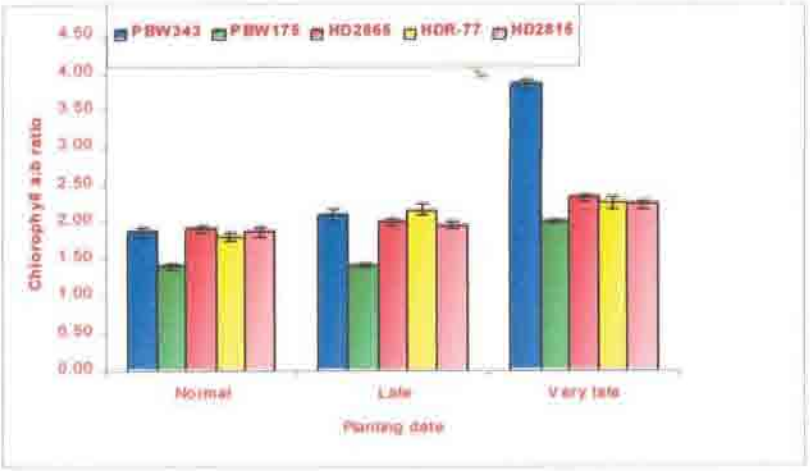
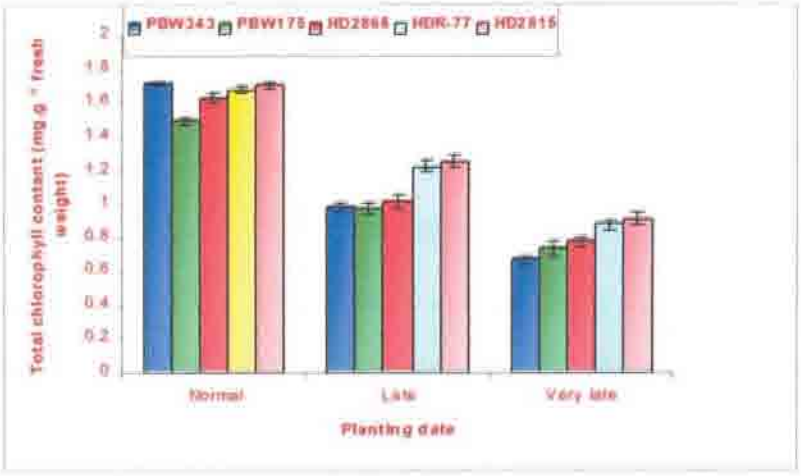
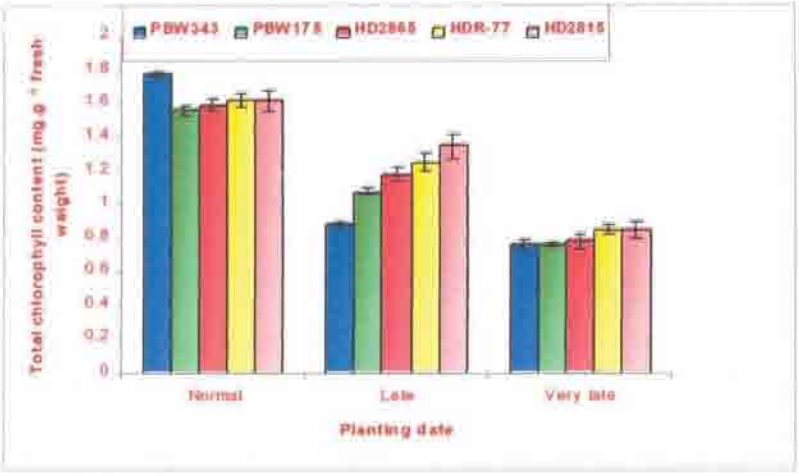


Fig. 20a, b and c. Chlorophyll a: b ratio in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively.

a



b



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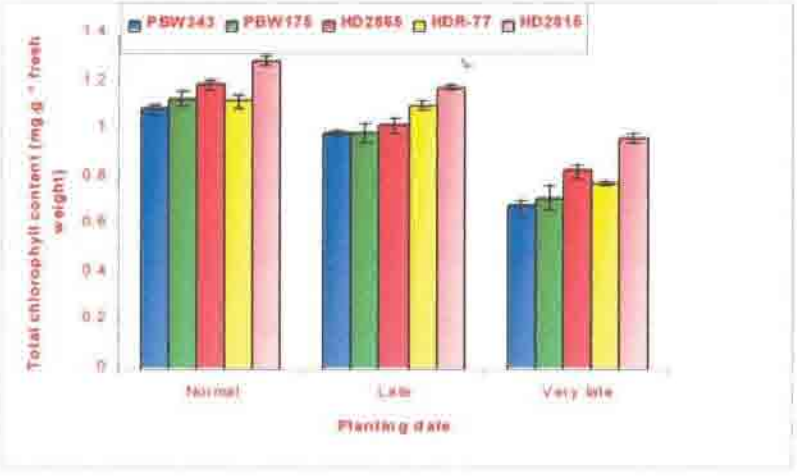


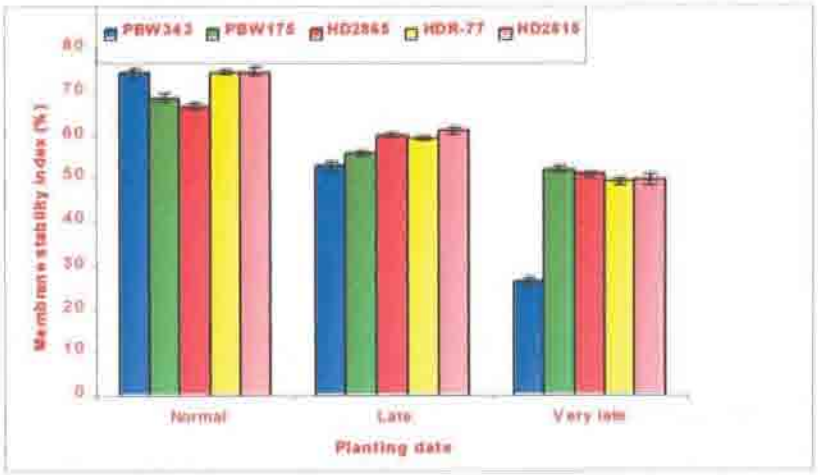
Fig. 21a, b and c. Total chlorophyll content (mg g^{-1} fresh weight) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

recorded under late and very late planting compared to normal. Under normal planting highest total chlorophyll content was recorded in PBW 343 at vegetative and anthesis stage, HD 2815 at 15 days after anthesis. Under late planting highest total chlorophyll content was recorded in HDR-77 at vegetative stage, HD 2815 at anthesis and at 15 days after anthesis, while under very late planting highest total chlorophyll content was recorded in HD 2815 at all growth stages. Maximum reduction in total chlorophyll content under late planting compared to normal was recorded in PBW 343 i.e., 42 and 51% at vegetative and anthesis stage respectively, while in HD 2865 at 15 days after anthesis i.e., 14%. Under very late planting highest reduction in total chlorophyll content compared to normal planting was recorded in PBW 343 at all stages of growth i.e., 61, 57 and 38% respectively. However minimum reduction in total chlorophyll content under late planting conditions was recorded in HD 2815 and HDR-77 at all growth stages (Fig. 21a, b and c).

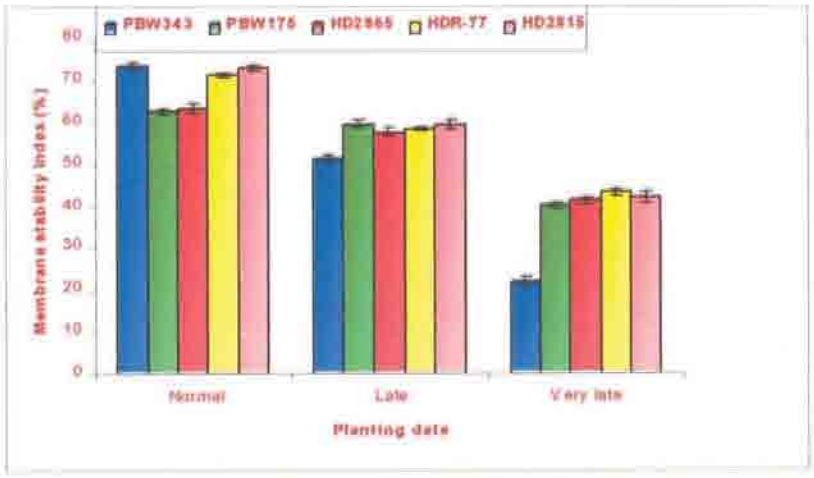
4.3.1.2 Membrane Stability Index

Significant reduction in membrane stability index was clearly observed under late and very late plantings compared to normal. Highest value for MSI under normal planting was recorded in PBW 343 at vegetative and anthesis stage and in HD 2815 at 15 days after anthesis, while under late planting highest MSI value was recorded in HD 2815 at all stages of growth. Under very late planting highest MSI value was recorded in PBW 175 at vegetative stage, HDR-77 at anthesis stage and HD 2815 at 15 days after anthesis. However, maximum reduction in MSI under late planting was recorded in PBW 343 i.e., 29, 30 and 19% respectively, while under very late planting

a



b



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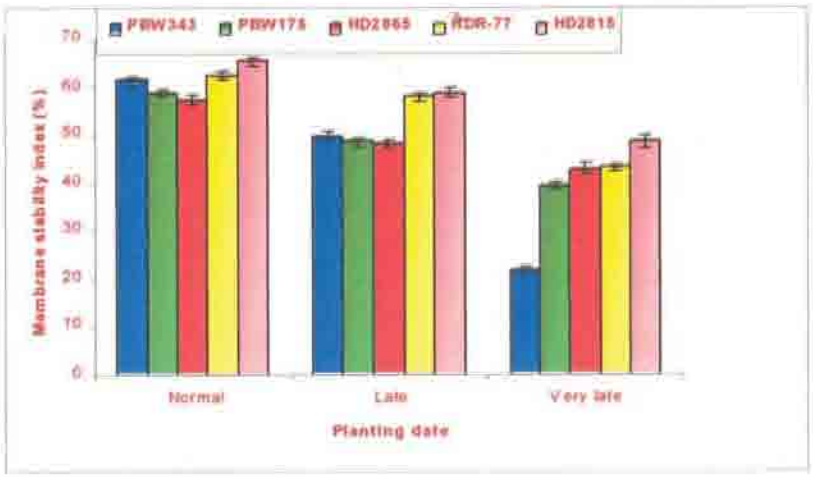


Fig. 22a, b and c. Membrane stability index (%) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

PBW 343 showed maximum reduction in MSI i.e., 65, 70 and 64% respectively (Fig. 22a, b and c).

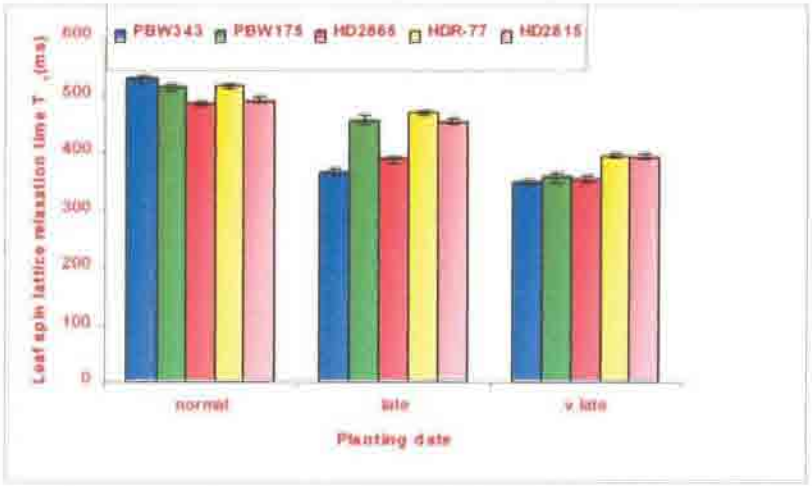
4.3.1.3 Leaf Water Spin Lattice Relaxation Time (T_1)

Leaf water spin lattice relaxation time (T_1) significantly decreased under late and very late plantings compared to normal. Highest T_1 value under normal planting was recorded at vegetative stage in PBW 343, at anthesis in PBW 175 and at 15 days after anthesis in HDR-77, while under late and very late plantings highest T_1 value was recorded in HDR-77 at vegetative stage and at 15 days after anthesis and in HD 2815 at anthesis stage. However, maximum reduction in T_1 value under late and very late plantings was recorded in PBW 343 i.e., 31 and 34% respectively at vegetative stage, 39 and 48% respectively at anthesis stage and 22 and 33% respectively at 15 days after anthesis. Minimum reduction under late planting condition was recorded in HD 2815 and HDR-77 at all stages of growth (Fig. 23a, b and c).

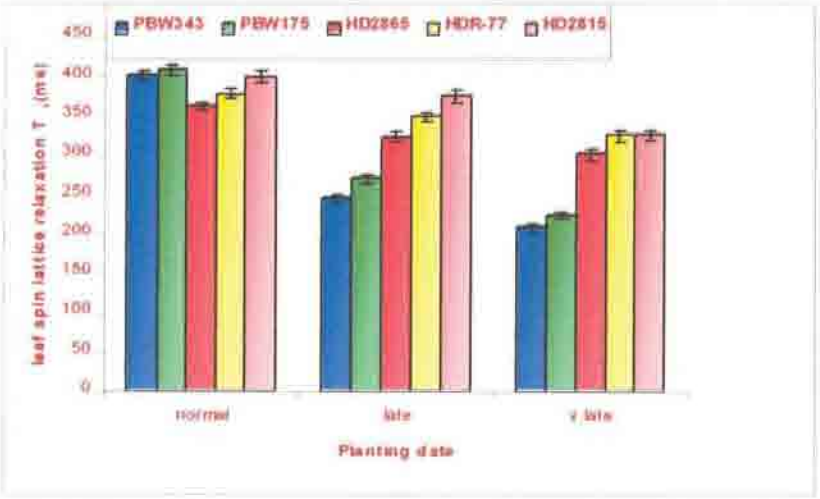
4.3.1.4 Photosynthetic Rate

There was significant reduction in photosynthetic rate under late and very late planting compared to normal. Under normal planting highest photosynthetic rate was recorded in PBW 343 at vegetative stage and at 15 days after anthesis, HD 2865 at anthesis stage, while under late planting highest Pn rate was recorded in PBW 343 at vegetative stage and in PBW 175 at anthesis and in HD 2815 at 15 days after anthesis. Under very late planting highest Pn rate was recorded in HDR-77 at vegetative stage and in HD 2815 at anthesis and at 15 days after anthesis. Maximum reduction in photosynthetic rate was recorded in PBW 343 at vegetative stage and at 15 days after anthesis i.e., 15, 13% respectively, at anthesis in HD 2865 i.e.,

a



b



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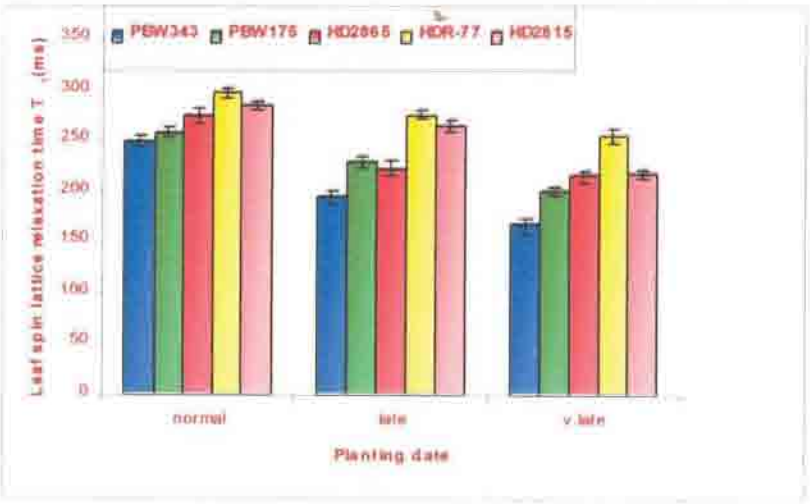
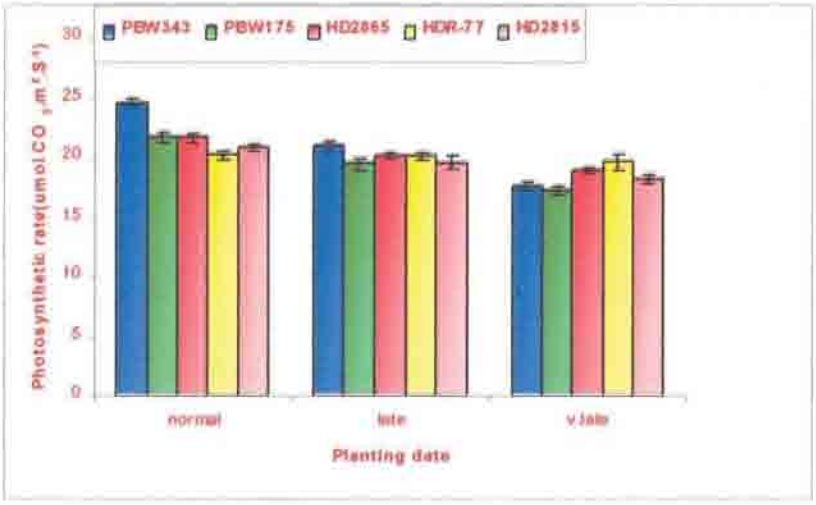


Fig. 23a, b and c. Leaf spin lattice relaxation time T_1 (ms) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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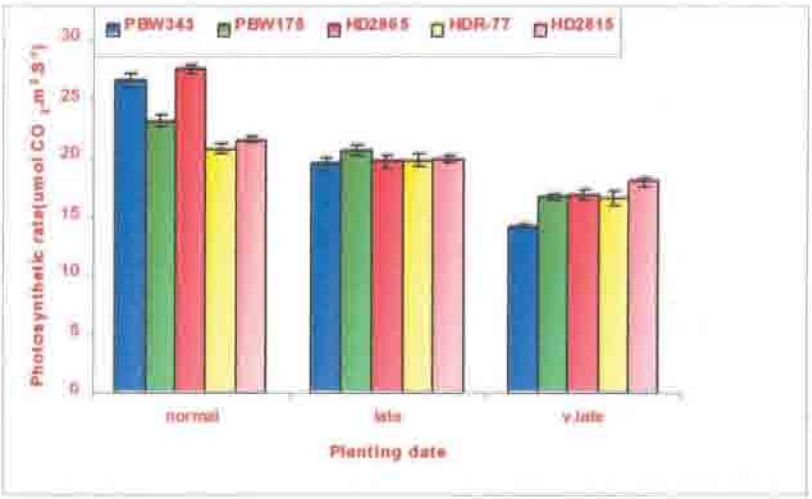

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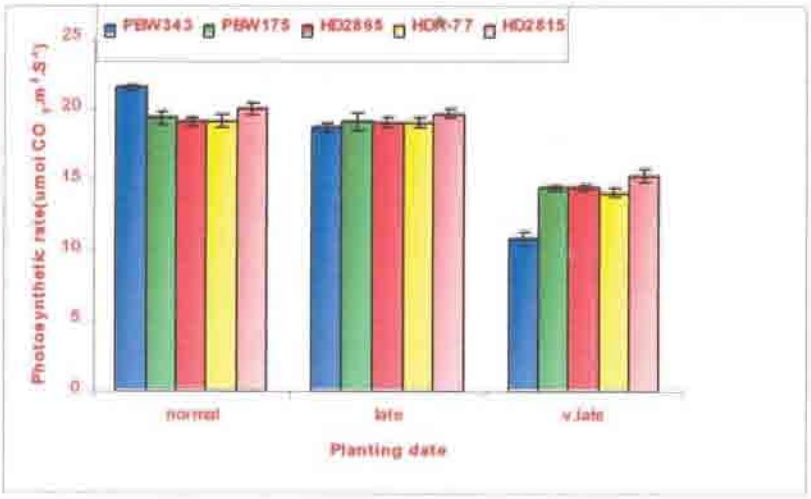


Fig. 24a, b and c. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

28%, while under very late planting maximum reduction compared to normal planting was recorded in PBW 343 i.e., 29, 47 and 50% respectively (Fig. 24a, b and c).

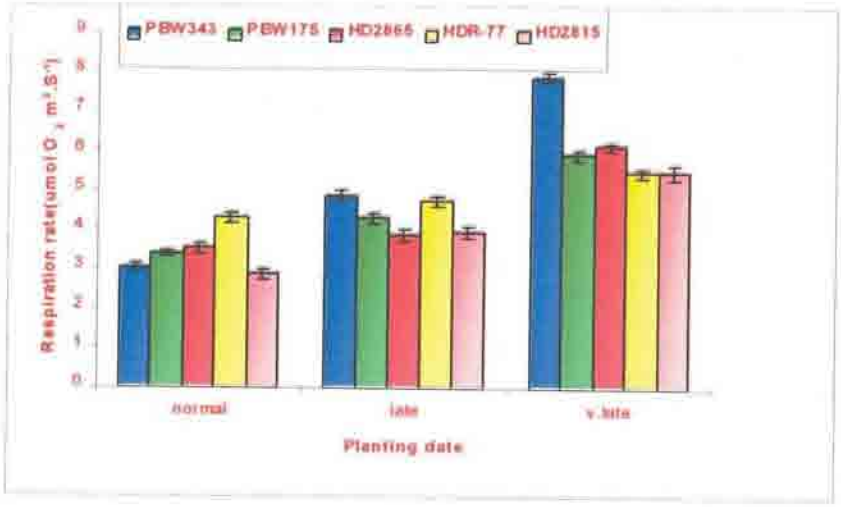
4.3.1.5 Respiration Rate

Significant increase in respiration rate was recorded under late and very late planting compared to normal. Highest respiration rate under normal planting was recorded in HDR-77 at all stages of growth, while under late and very late planting highest respiration rate was recorded in PBW 343 at all stages of growth (Fig. 25a, b and c).

4.3.1.6 Water Potential

Reduction in water potential under late and very late planting was recorded in all genotypes. Under normal planting highest water potential value was recorded in PBW 175 at anthesis stage, in PBW 343 at 7 days after anthesis and in HD 2815 at 14 days after anthesis, while under late and very late plantings highest water potential value was recorded in HD 2815 at all stages of growth. Maximum reduction in water potential under late planting compared to normal was recorded in PBW 175 at anthesis stage i.e., 18%, at 7 and 14 days after anthesis in PBW 343 i.e., 34 and 15% respectively. Under very late planting maximum reduction in water potential compared to normal planting was recorded in PBW 343 i.e., 26, 45 and 36% respectively (Table 20).

a



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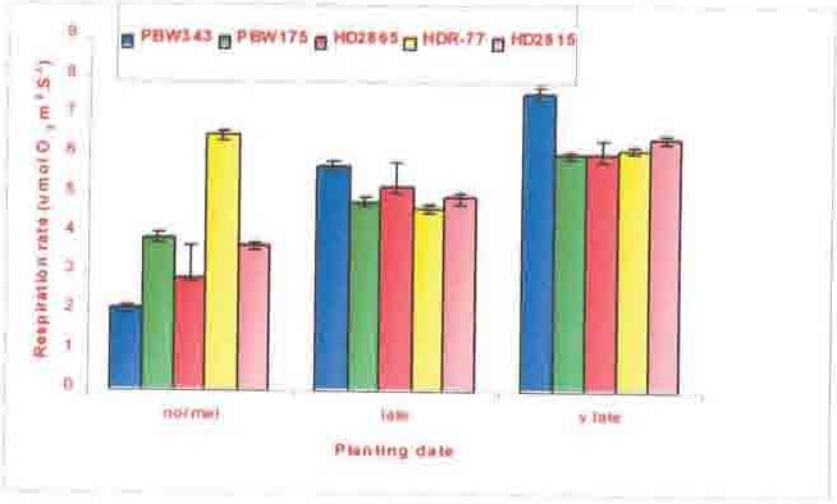

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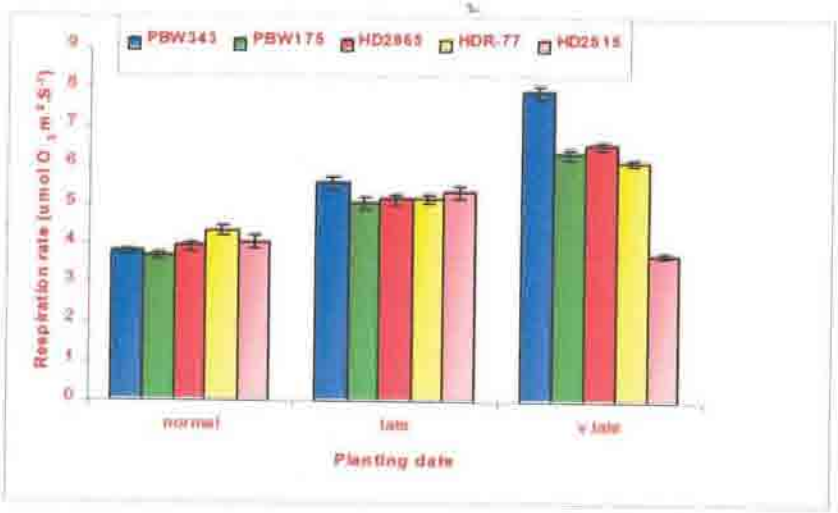


Fig. 25a, b and c. Respiration rate (µmol O_2 m^{-2} S^{-1}) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

Table 20a,b and c. Water potential (MPa) in five wheat genotypes under normal, late and very late planting, at anthesis stage, 7 and 14 days after anthesis respectively.

(a)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-1.97	-2.3	-2.49
PBW175	-1.87	-2.21	-2.28
HD2865	-1.9	-2.05	-2.31
HDR-77	-2.11	-2.34	-2.35
HD2815	-1.92	-2.05	-2.15
CD at 5%	0.06	0.08	0.14

(b)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-1.47	-1.97	-2.13
PBW175	-1.59	-1.84	-2.13
HD2865	-1.84	-1.9	-2.23
HDR-77	-1.68	-1.84	-2.04
HD2815	-1.76	-1.84	-2.17
CD at 5%	0.05	0.07	0.12

(c)

	Water potential		
Genotypes	**Normal**	**Late**	**Very Late**
PBW343	-1.97	-2.27	-2.68
PBW175	-1.88	-2.13	-2.43
HD2865	-1.86	-2.04	-2.14
HDR-77	-1.9	-2.07	-2.21
HD2815	-1.81	-2.01	-2.18
CD at 5%	0.05	0.07	0.12

4.3.1.7 Antioxidant enzymes

4.3.1.7.1 Superoxide Dismutase

SOD activity increased gradually at all stages of growth in all genotypes under late and very late plantings. Under normal planting at vegetative and anthesis stages highest SOD activity was recorded in HD 2815, at 15 days after anthesis in PBW 175, while under the late planting HD2815 showed highest value at all stages of growth. However, under very late planting highest values at vegetative stage was recorded in HDR-77, at anthesis stage in HD 2815 and at 15 days after anthesis in PBW 343 (Fig. 26a, b and c).

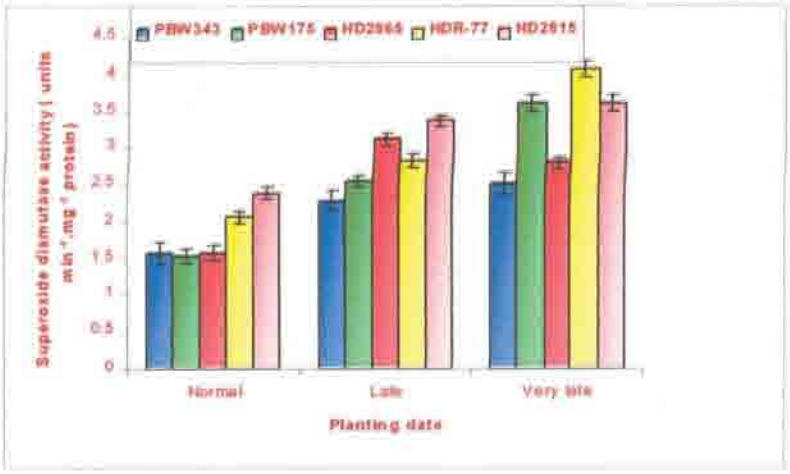
4.3.1.7.2 Catalase

Significant increase in catalase activity with delay in planting was recorded at all the growth stages in all the genotypes except PBW 343. Under normal planting at vegetative stage maximum activity was recorded in HD 2865, at anthesis in HD 2815 and at 15 days after anthesis in HDR-77. Under late planting highest activity was recorded at all stages in HD 2815. Under very late planting maximum activity at vegetative stage was recorded in HD 2865, at anthesis and at 15 days after anthesis in HD 2815. However, maximum activity under all planting for all genotypes was observed at anthesis stage (Fig. 27a, b and c).

4.3.1.7.3 Ascorbate Peroxidase

Significant increase in APX activity was observed under late and very late plantings at anthesis and at 15 days after anthesis. However under normal planting maximum activity was recorded at vegetative stage and HD 2865 showed highest activity at all the growth stages. Under late planting

a



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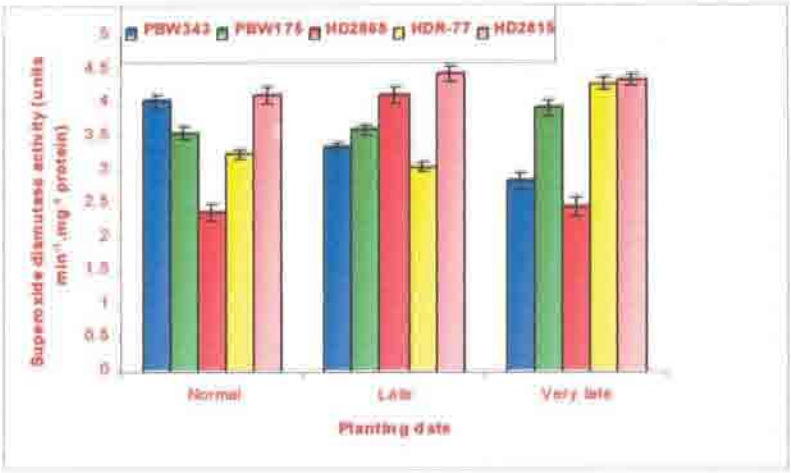

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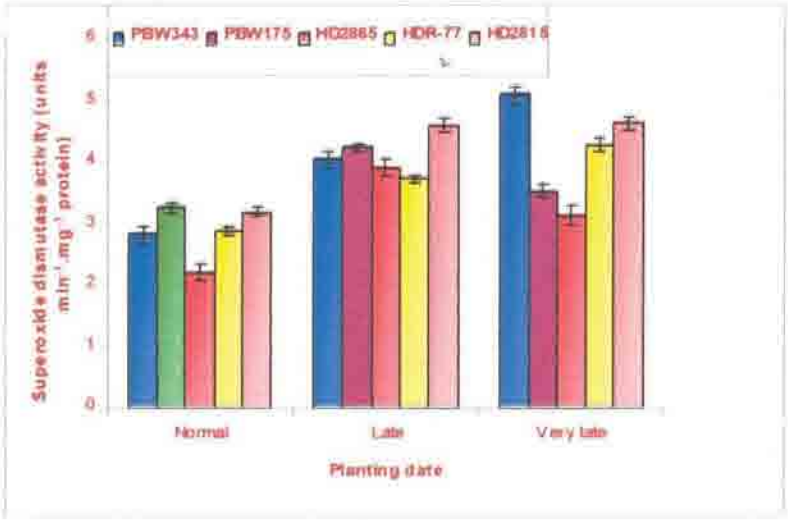
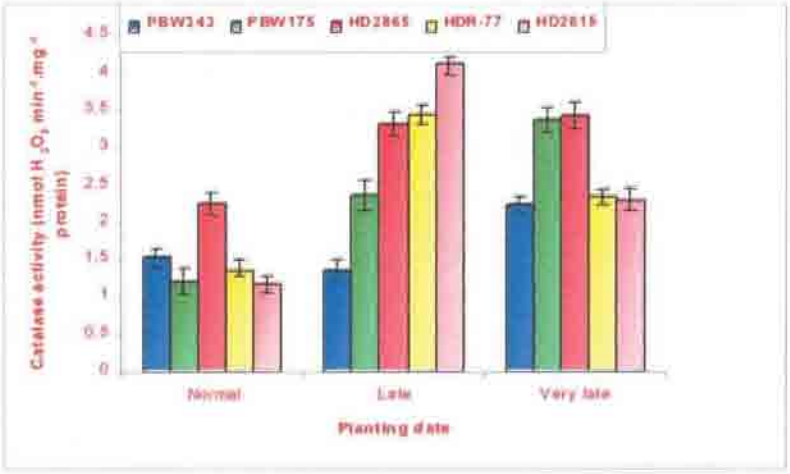


Fig. 26a, b and c. Superoxide dismutase activity (unit's min^{-1} mg^{-1} protein) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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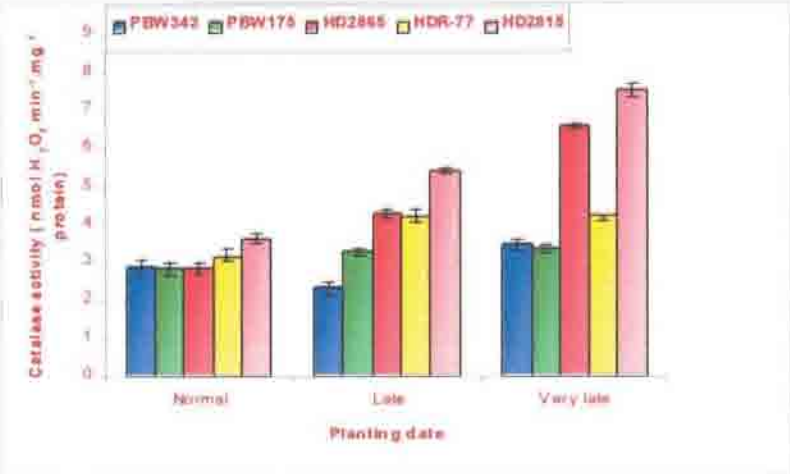

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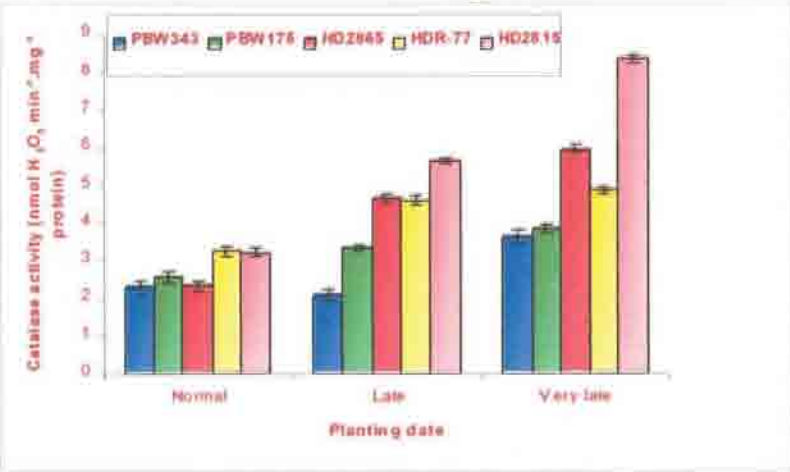
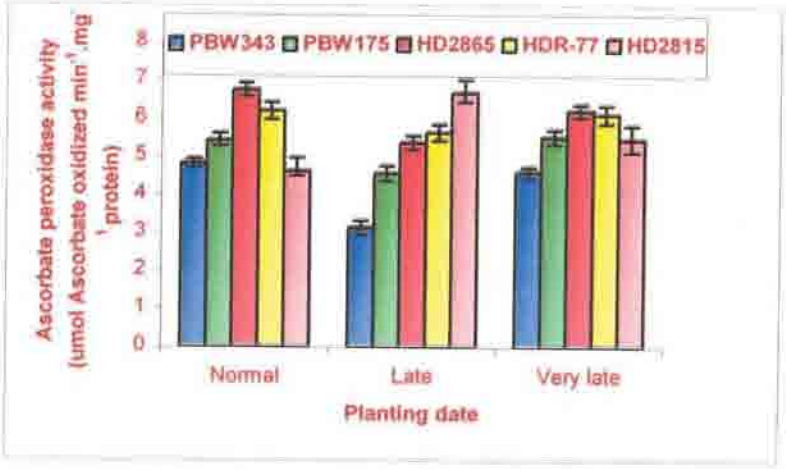


Fig. 27a, b and c. Catalase activity (nmol H_2O_2 min^{-1} mg^{-1} protein) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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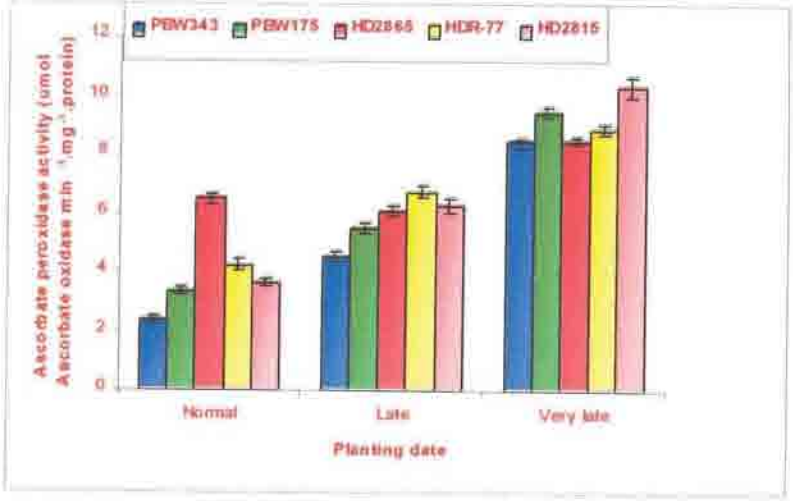

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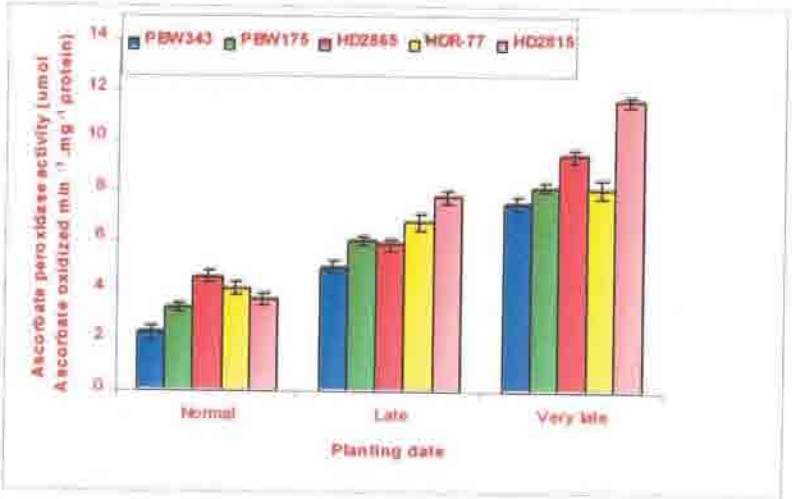


Fig. 28a, b and c. Ascorbate peroxidase activity (µmol ascorbate oxidized min^{-1} mg^{-1} protein) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively.

maximum activity was recorded at vegetative stage and at 15 days after anthesis in HD 2815 and at anthesis stage in HDR-77. However under very late planting maximum activity at all stages were recorded in HD 2815 (Fig. 28a, b and c).

4.3.1.7.4 Glutathione Reductase

GR activity decreased gradually and significantly by delayed planting at vegetative stage and at 15 days after anthesis. However, at anthesis stage GR activity increased under late planting but decreased under very late planting. Highest value for GR under normal planting at vegetative stage was recorded in HD 2865, at anthesis in HD 2815 and at 15 days after anthesis in PB W343, while in the late planting highest activity at vegetative stage was recorded in HD 2815, at anthesis stage in HDR-77, and at 15 days after anthesis in HD 2865. Under very late planting highest activity at vegetative and anthesis stages were recorded in HDR-77 and at 15 days after anthesis in HD 2815 (Fig. 29a, b and c).

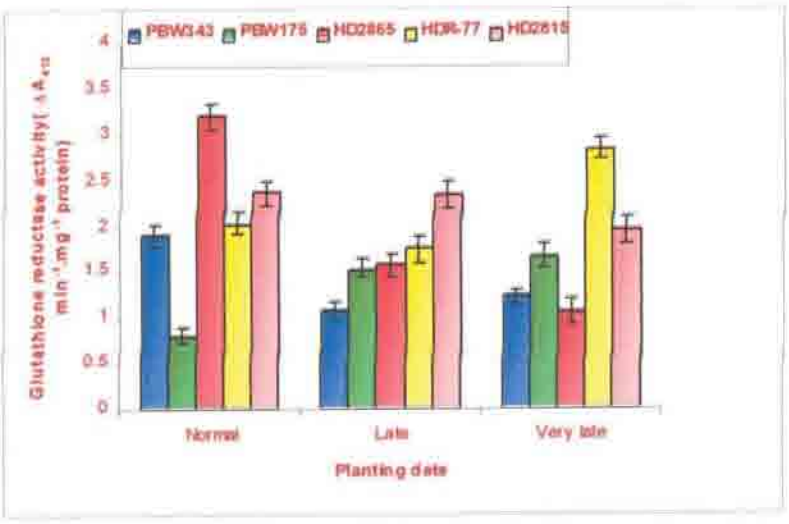
4.3.1.7.5 Peroxidase

Peroxidase activity decreased significantly under late plantings at all growth stages. Under normal planting highest activity was recorded in PBW 175, while under late planting highest activity was recorded in HD 2815 at all the growth stages. Under very late planting highest activity at vegetative stage was recorded in PBW 343, at anthesis stage and at 15 days after anthesis in HD 2815 (Fig. 30a, b and c).

4.3.2 Absolute Growth Rate

Observation on absolute growth rate showed that grain growth rate decreased significantly in all genotypes under late and very late planting

a



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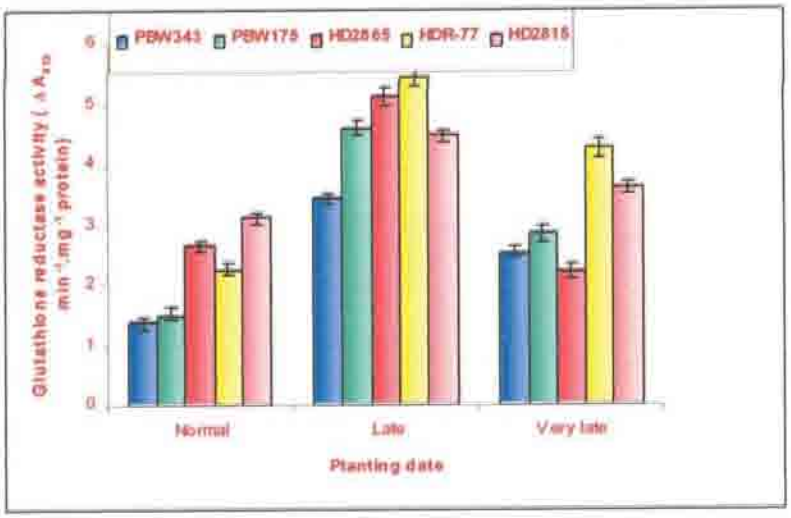

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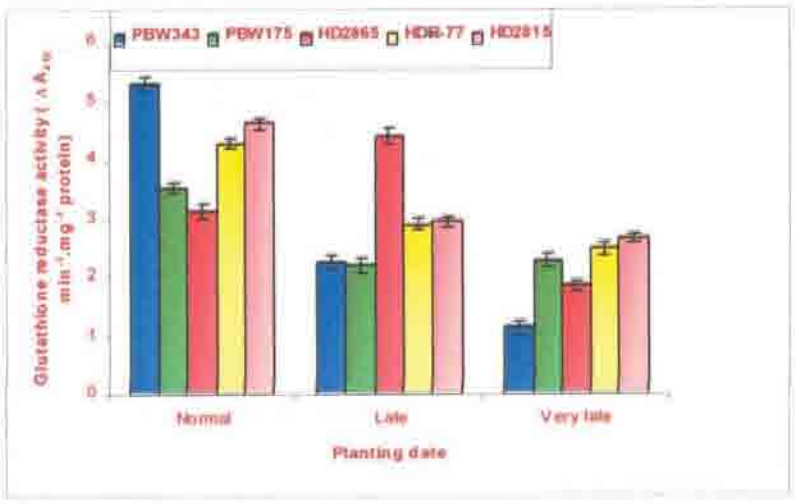
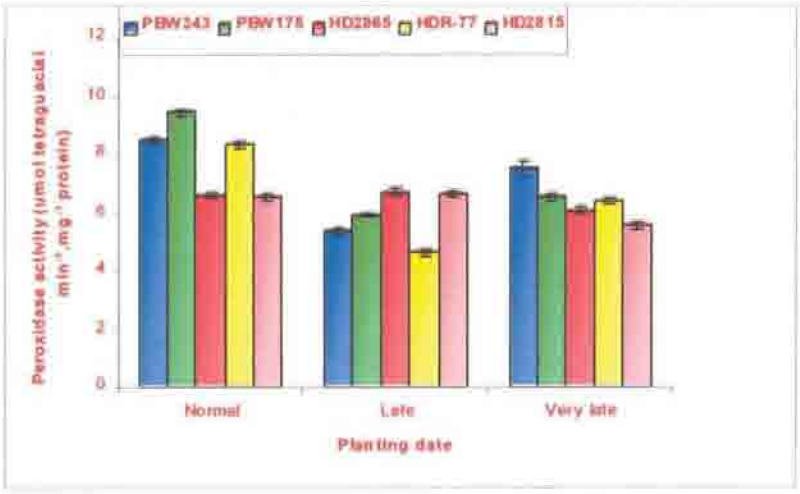


Fig. 29a, b and c. Glutathione reductase activity (ΔA_{412} min^{-1} mg^{-1} protein) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively

a



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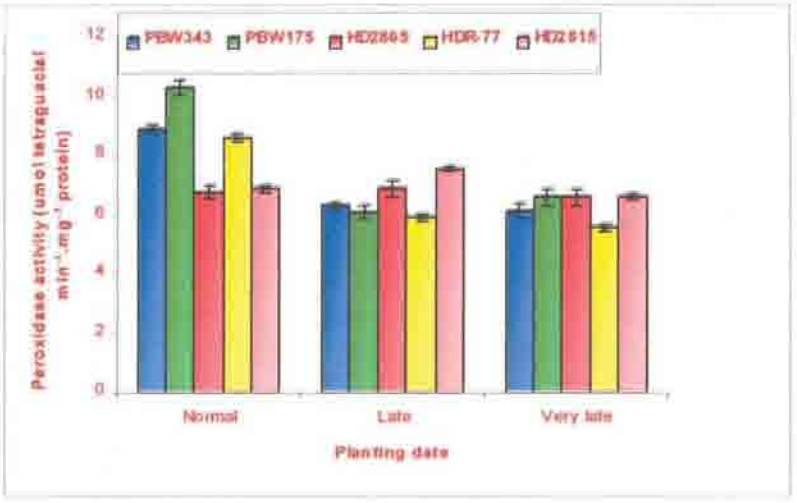

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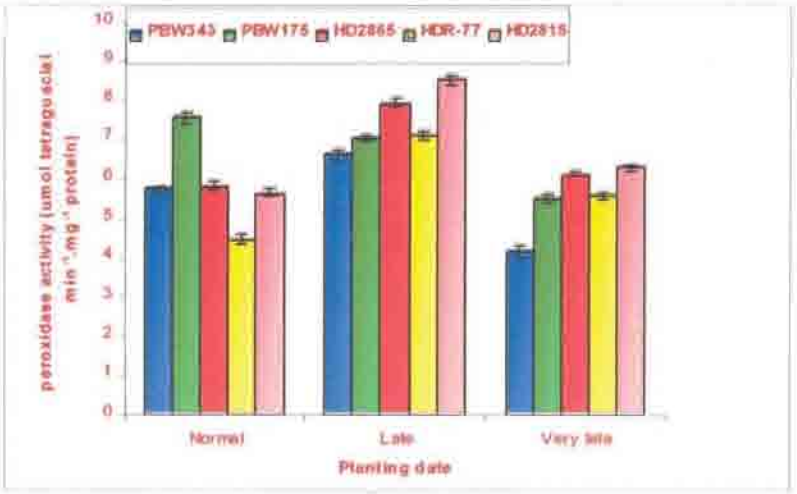

Fig 30a, b and c. Peroxidase activity (µmol tetraguacial min^{-1} mg^{-1} protein) in five wheat genotypes under normal, late and very late planting, at vegetative stage, anthesis and 15 days after anthesis respectively.

Table 21. Absolute growth rate (mg dry weight/day) in five wheat genotypes under normal, late and very late planting

Genotypes	Normal	Late	Very Late
PBW343	12.10	9.50	8.30
PBW175	10.90	9.00	8.00
HD2865	12.60	8.80	7.00
HDR-77	10.50	10.20	1.00
HD2815	10.30	9.80	9.70
CD at 5%	1.20	5.30	1.30

Table 22. Stem reserve mobilization (%) in five wheat genotypes under normal, late and very late planting

Genotypes	Normal	Late	Very Late
PBW343	64.75	64.87	68.11
PBW175	60.23	70.23	73.19
HD2865	65.87	71.09	77.08
HDR-77	62.57	63.44	71.73
HD2815	58.97	64.55	70.39
CD at 5%	1.23	1.87	2.61

compared to normal. However, HD 2865 and PBW 343 showed high per cent of reduction under late planting conditions compared to normal. HDR-77 and HD 2815 showed less reduction compared to other genotypes under late planting conditions (Table 21).

4.3.3 Stem Reserve Mobilization

The value for stem reserve mobilization increased significantly under late and very late planting compared to normal. At all the plantings highest stem reserve mobilization value was recorded in HD 2865 and maximum increase in stem reserve mobilization value under late planting conditions compared to normal was recorded in HD 2815 (Table 22).

4.3.4 Yield and Yield attributes

4.3.4.1 Total Biomass per Plant

Total biomass per plant decreased significantly under late and very late planting compared to normal. Under normal planting highest biomass per plant was recorded in PBW 343. Under late and very late plantings highest value was recorded in HD 2815. However, maximum reduction in total biomass per plant was recorded in PBW 343 under late planting compared to normal i.e., 36%. Under very late planting maximum reduction was recorded in PBW 175 i.e., 66%, while minimum reduction under late and very late plantings was recorded in HDR-77 and HD 2815 (Fig. 31).

4.3.4.2 Grain Yield per Plant

Reduction in grain yield per plant was clearly seen under late and very late planting compared to normal. Under normal planting highest value for grain yield was recorded in PBW 175 at normal and late planting, while under very late planting genotypes showed no significant difference in grain yield per

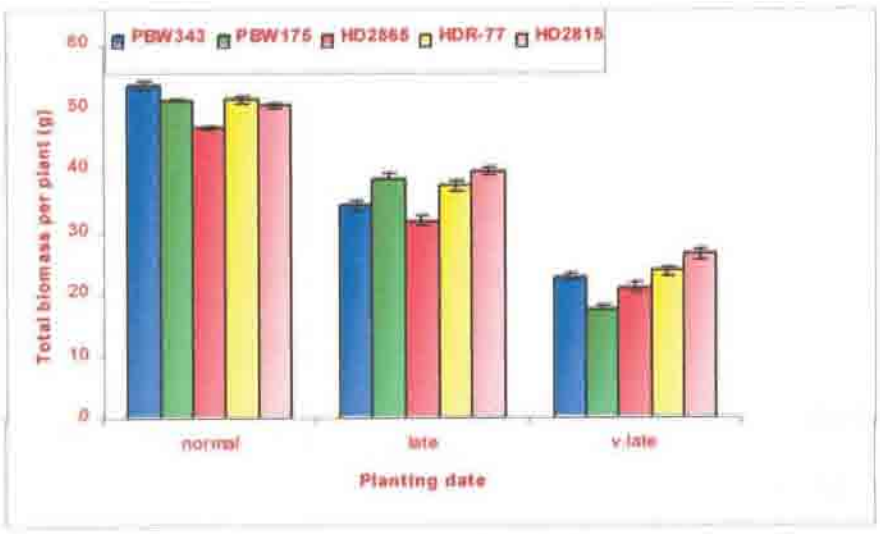

Fig. 31. Total biomass per plant (g) in five wheat genotypes under normal, late and very late planting

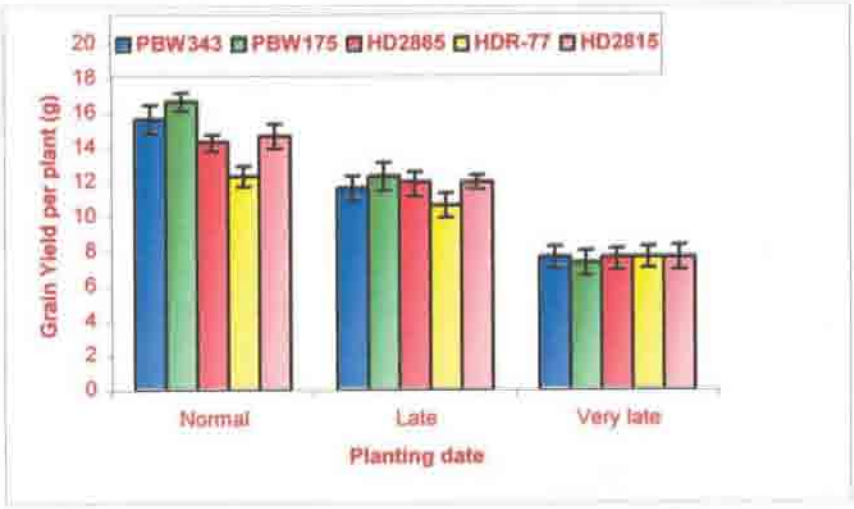

Fig. 32. Grain yield per plant (g) in five wheat genotypes under normal, late and very late planting

plant. Maximum reduction in grain yield under late and very late planting compared to normal was recorded in PBW 175 i.e., 26, 56%, while minimum reduction was recorded in HDR-77 under late and very late planting compared to normal (Fig. 32).

4.3.4.3 Grain Number and Grain Weight per Ear

Significant reduction in grain number and grain weight per ear was clearly seen under late and very late plantings compared to normal. Under normal and very late planting maximum number of grain per ear was recorded in HDR-77, while under late planting in HD 2815 (Fig. 33). Maximum reduction in grain number per ear was recorded in PBW 343 under late and very late plantings compared to normal i.e., 16, 28% respectively, while minimum reduction was recorded in HD 2865 under late planting conditions. Maximum grain weight per ear was recorded in PBW 343 under normal planting, HD 2815 under late planting and no significant difference in grain weight per ear was recorded between all genotypes under very late planting. Maximum reduction in grain weight per ear was recorded in PBW 343 under late and very late plantings compared to normal i.e., 25, 39% respectively, while minimum reduction was recorded in HD 2815 under late planting and HD 2865 under very late planting (Fig. 34).

4.3.4.4 1000 Grain Weight

1000 grain weight was significantly reduced under late and very late plantings compared to normal and highest value for 1000 grain weight was recorded in HD 2815 under normal, late and very late plantings. Maximum reduction in 1000 grain weight under late and very late planting compared to

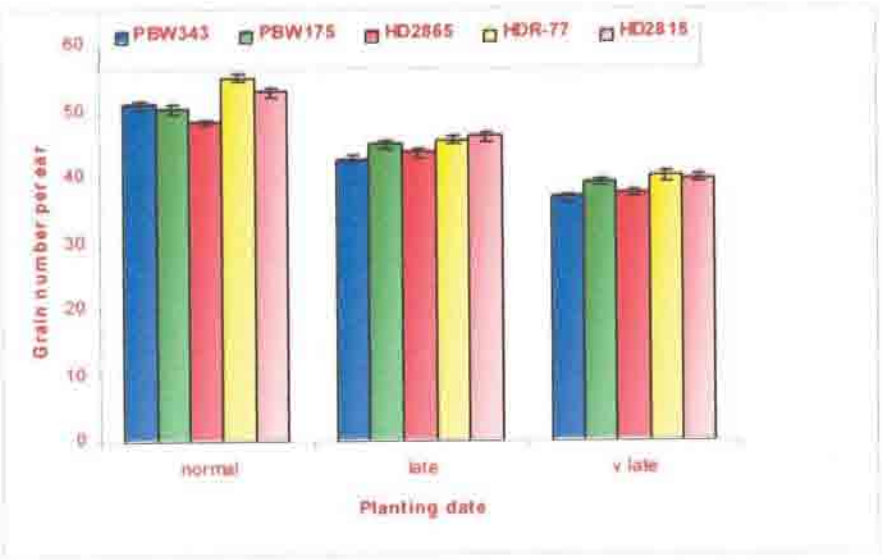

Fig. 33. Grain number per ear in five wheat genotypes under normal, late and very late planting

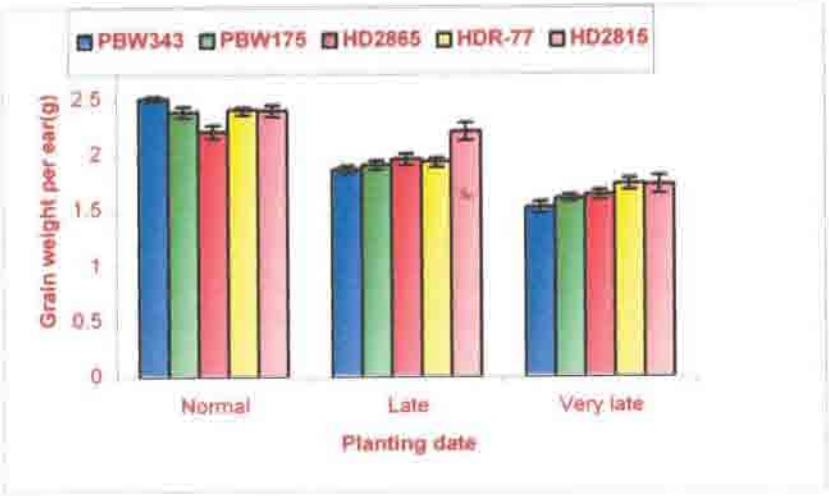

Fig. 34. Grain weight per ear (g) in five wheat genotypes under normal, late and very late planting

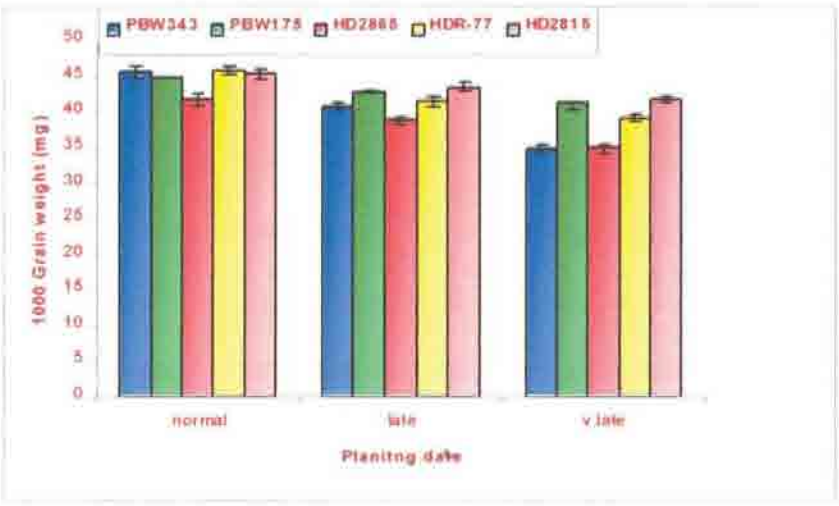

Fig. 35. 1000 Grain weight (g) in five wheat genotypes under normal, late and very late planting

normal was recorded in PBW 343 i.e., 11, 24%, while minimum reduction was recorded in HD 2815 (Fig. 35).

4.4 EXPERIMENT-4

4.4.1 Physiological and Biochemical Parameters

4.4.1.1 Chlorophyll Content

There was significant reduction in chlorophyll-a content in both genotypes when grown under heat stress treatment, PBW 343 showed higher chlorophyll-a content under normal temperature at all growth stages, while C 306 showed higher chlorophyll-a content at anthesis stage under heat stress treatment and PBW 343 showed higher content of chlorophyll-a at vegetative stage and at 15 days after anthesis (Fig. 36a). Significant reduction in chlorophyll-b content was recorded in both genotypes under heat stress treatment. Highest chlorophyll-b content was recorded in C 306 at anthesis stage and at 15 days after anthesis under normal treatment and chlorophyll-b content was higher in PBW 343 at vegetative stage, while under heat stress treatment C 306 showed highest chlorophyll-b content at all stages of growth (Fig. 36b). There was significant increase in chlorophyll a:b ratio under heat stress treatment compared to normal temperature and maximum chlorophyll a:b ratio was recorded in PBW 343 at all stages of growth (Fig. 36c). Total chlorophyll content was significantly reduced under heat stress treatment compared to normal temperature in both genotypes at all growth stages. C 306 showed 16.5, 22.8 and 47.5% reduction in chlorophyll content at vegetative stage, anthesis and 15 days after anthesis respectively. While PBW 343 showed 34.5, 29.2 and 60.9% reduction respectively. Plant grown under normal temperature showed highest chlorophyll content at 15 days after

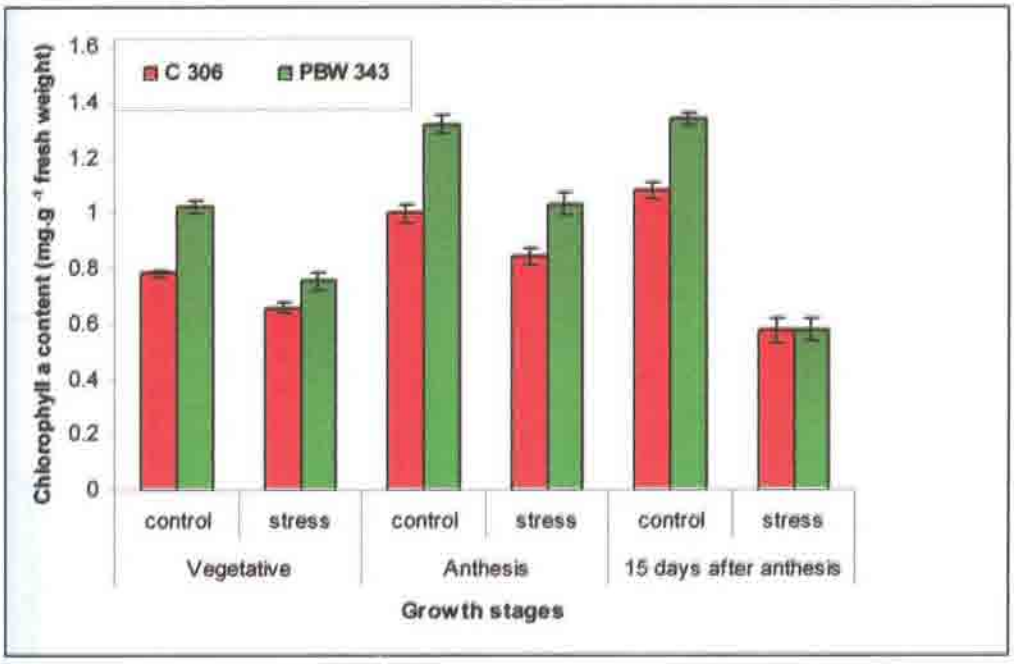

Fig. 36a. Chlorophyll a content (mg.g^{-1} fresh weight) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

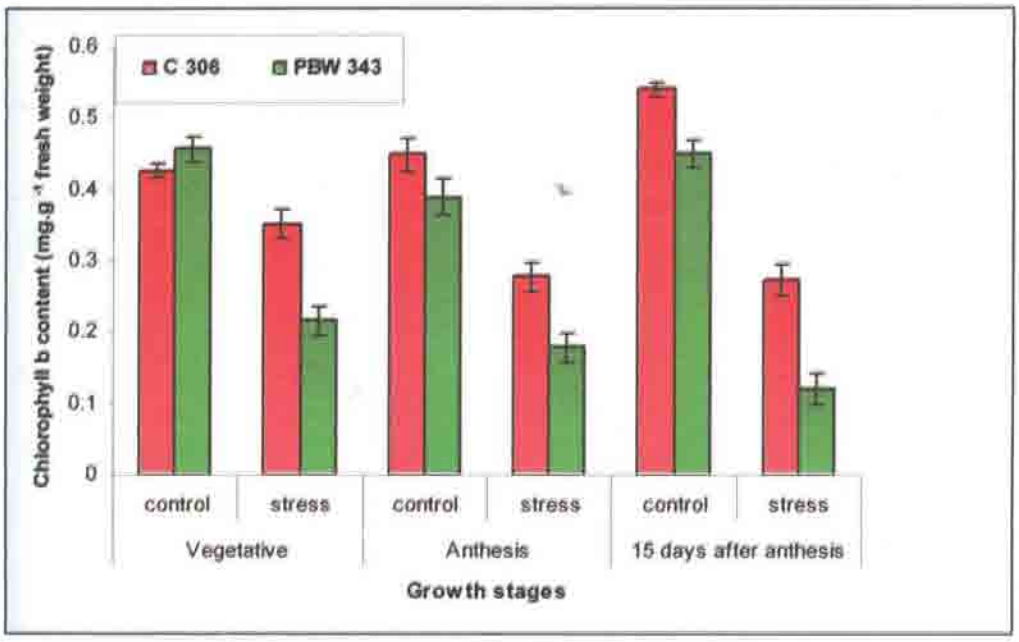

Fig. 36b. Chlorophyll b content (mg.g^{-1} fresh weight) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

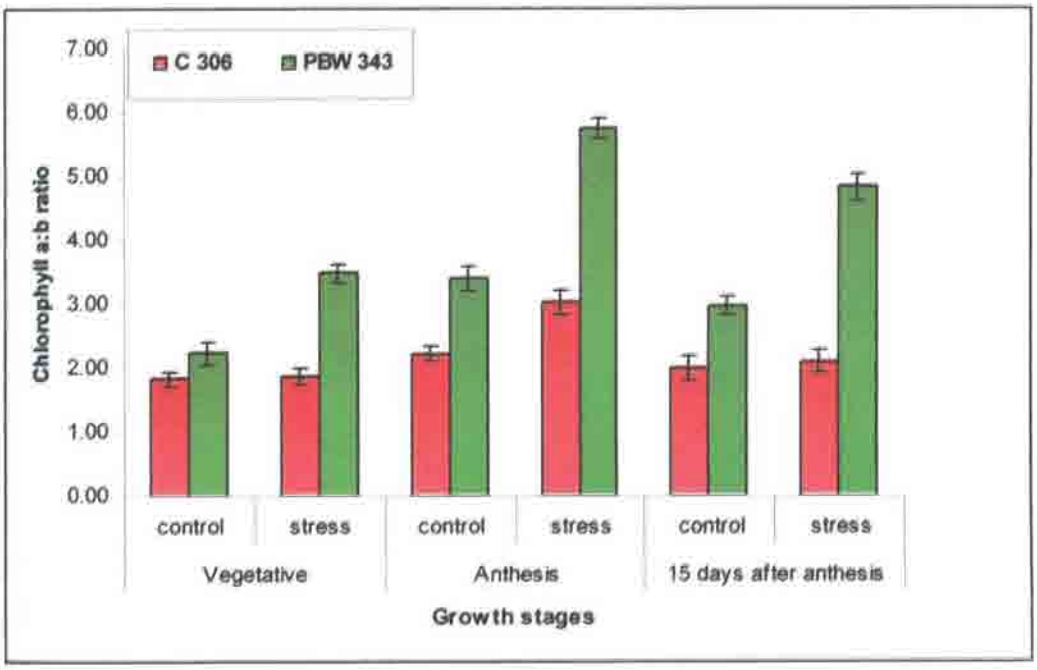

Fig. 36c. Chlorophyll a:b ratio in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

anthesis in both genotypes, while both genotypes under heat stress conditions showed highest chlorophyll content at anthesis stage (Fig. 37).

4.4.1.2 Membrane Stability Index

Observations on membrane stability index revealed that there was significant reduction in MSI in both genotypes at all stages of growth under heat stress treatment compared to normal temperature. However, C 306 showed better performance and more stability under heat stress treatment at all stages compared to PBW 343 and maximum reduction in membrane stability index was recorded at vegetative stage (Fig. 38). The per cent reduction in C 306 under heat stress treatment compared to normal temperature at vegetative stage, anthesis and 15 days after anthesis were 13, 13 and 10% respectively, while PBW 343 showed 35, 33 and 29% respectively.

4.4.1.3 Leaf Water Spin Lattice Relaxation Time (T_1)

Leaf water spin lattice relaxation time (T_1) showed highest value in both genotypes under both treatments at vegetative stage. Significant reduction in relaxation time T_1 was recorded in both genotypes under heat stress treatment and more reduction was recorded in PBW 343 at all stages of growth compared to C 306. However, maximum reduction in leaf spin lattice relaxation time T_1 in C 306 was recorded at anthesis stage, while in PBW 343 maximum reduction was recorded at 15 days after anthesis. The per cent reduction in leaf spin lattice relaxation time (T_1) in C 306 under heat stress treatment was 5, 10 and 9% respectively, while PBW 343 showed 24, 18 and 33% respectively (Fig. 39).

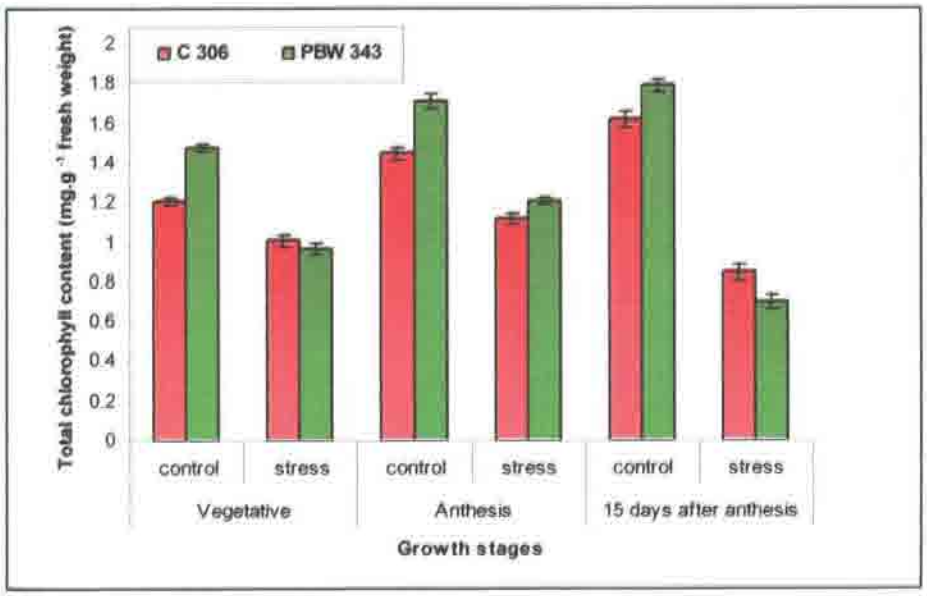

Fig. 37. Total chlorophyll content (mg.g^{-1} fresh weight) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

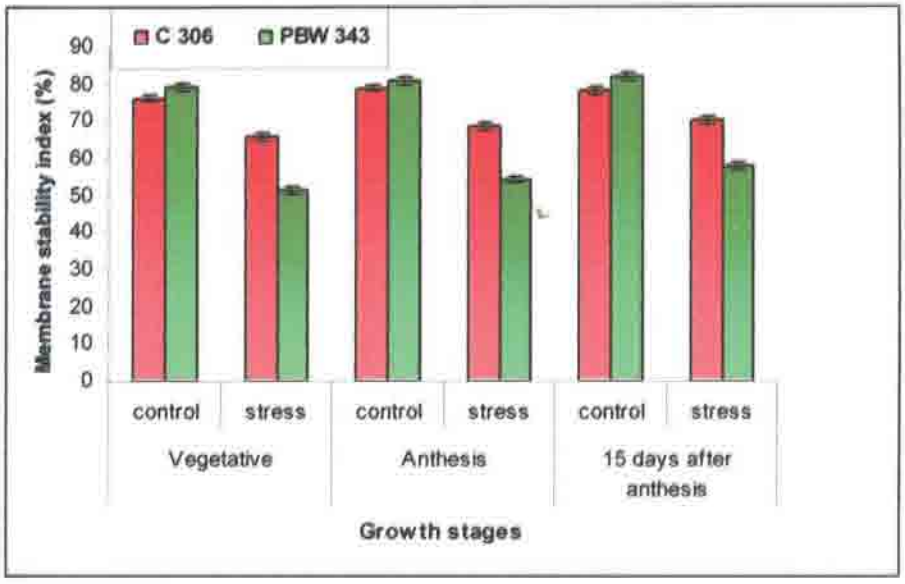

Fig. 38. Membrane stability index (%) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

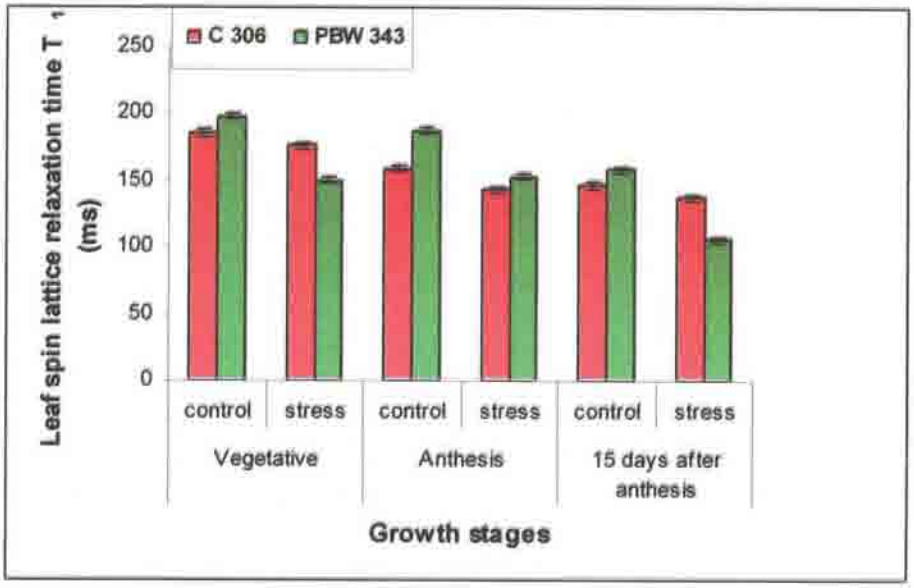

Fig. 39. Leaf spin lattice relaxation time T_1 (ms) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

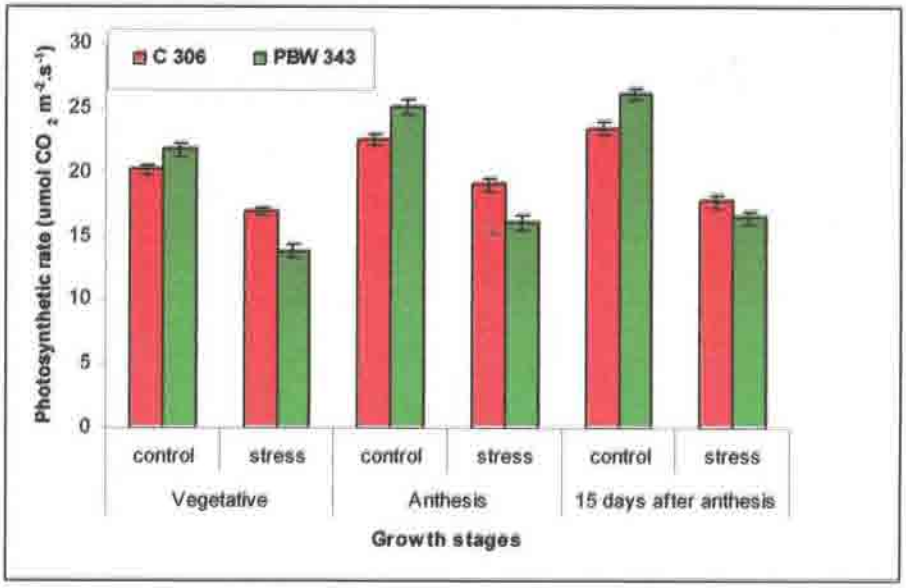

Fig. 40. Photosynthetic rate (µmol CO_2 $m^2.S^{-1}$)) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

4.4.1.4 Photosynthetic Rate

Highest photosynthetic rate in both genotypes under normal temperature was recorded at 15 days after anthesis. However, significant reduction in photosynthetic rate was recorded in both genotypes grown under heat stress treatment. C 306 showed 16, 15. 4 and 24.3% reduction under heat stress compared to normal temperature at vegetative stage, anthesis and 15 days after anthesis respectively, while PBW 343 showed 36.7, 35.9 and 37% respectively. However, maximum photosynthetic rate under heat stress was recorded at anthesis stage in C 306, while PBW 343 showed no significant difference in photosynthetic rate at anthesis stage and at 15 days after anthesis (Fig. 40).

4.4.1.5 Respiration Rate

Significant increase in respiration rate in C 306 and PBW 343 was recorded under heat stress treatment compared to normal temperature. Maximum respiration rate in both genotypes and under both growth conditions were recorded at 15 days after anthesis and C 306 showed comparatively lower respiration rate compared to PBW 343 which showed sharp increase in respiration rate under heat stress treatment (Fig. 41).

4.4.1.6 Rubisco Enzyme Activity

Maximum activity for Rubisco enzyme was recorded in both genotypes at anthesis stage under normal temperature; while both genotypes when exposed to heat stress treatment they showed maximum activity of the enzyme at vegetative stage. However, activity of the enzyme decreased significantly under heat stress and more reduction was recorded in PBW 343 at all stages of growth. C 306 showed 11.4, 29.9 and 25.7% reduction in

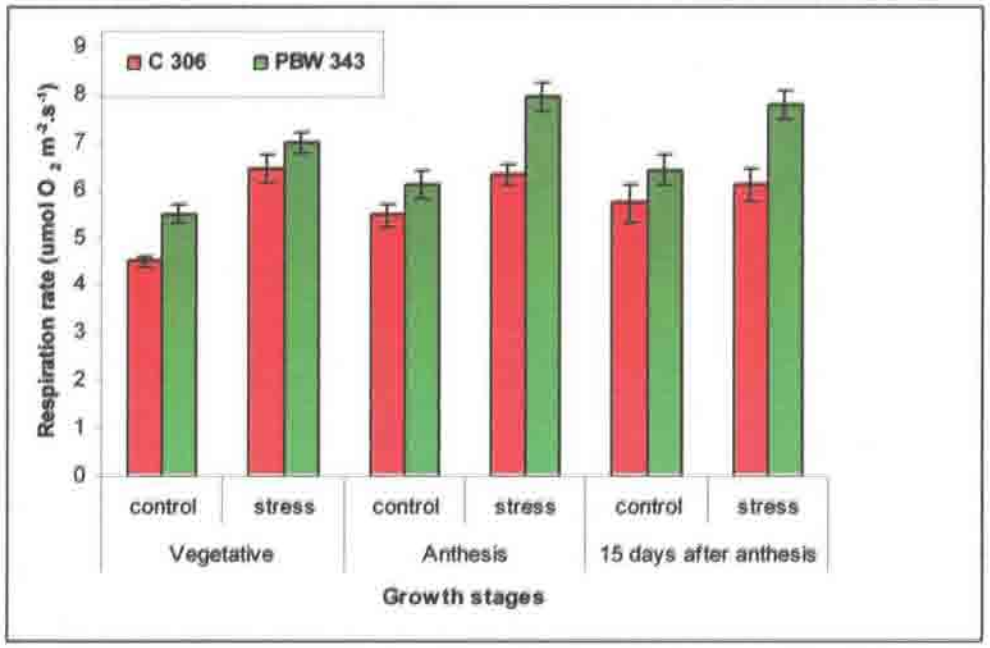

Fig. 41. Respiration rate (μmol O_2 $m^2.S^{-1}$)) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

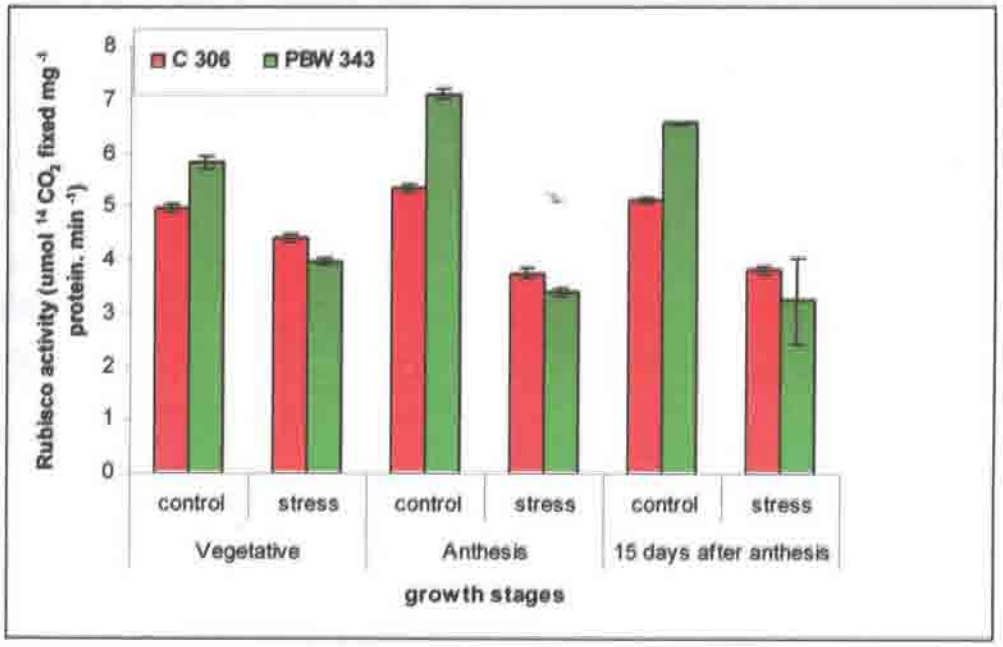

Fig. 42. Rubisco activity (μmol $^{14}CO_2$ fixed.min^{-1}.mg^{-1} protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

Table 23. Water potential (MPa) in C306 and PBW 343 under normal temperature and high temperature treatment at anthesis stage, 7 and 14 days after anthesis

	Water potential		
	Vegetative stage	**Anthesis stage**	**14days after anthesis**
C 306 at normal temperature	-1.88	-2.09	-2.31
C 306 at high temperature	-2.08	-2.17	-2.37
PBW 343 at normal temperature	-2.18	-2.21	-2.42
PBW 343 at high temperature	-2.57	-2.5	-2.84
CD at 5 %	0.278	0.306	0.403

Rubisco enzyme activity under heat stress compared to normal temperature at vegetative stage, anthesis and 15 days after anthesis respectively. While PBW 343 showed 31.7, 52.4 and 50.7% respectively (Fig. 42).

4.4.1.7 Leaf Water Potential

Maximum water potential was recorded at anthesis stages in both genotypes, under both treatments. However, significant reduction in water potential in leaf tissues was recorded at all stages of growth in both genotypes under heat stress treatment compared to normal temperature. More reduction in water potential was recorded in PBW 343 under heat stress condition compared to C 306, and C 306 sustain relatively high water potential even under heat stress condition, while PBW 343 showed drastic reduction in water potential at all stages (Table 23).

4.4.1.8 Hydrogen Peroxide

Significant increase in hydrogen peroxide content was recorded in both genotypes under heat stress treatment at all stages of growth and maximum hydrogen peroxide content was recorded in both genotypes at 15 days after anthesis at both treatments. However, more increase in hydrogen peroxide content due to heat stress treatment was recorded in PBW 343 compared to C 306 at all stages of growth (Fig. 43). The per cent increase in the amount of hydrogen peroxide was 10, 5 and 14% respectively in C 306, while it was 25, 37 and 27% respectively in PBW 343.

4.4.1.9 Antioxidant Enzyme Activity

There was significant increase in the antioxidant enzymes activities in both genotypes under heat stress conditions at all stages of growth, however significant increase was recorded in C 306 compared to PBW 343.

4.4.1.9.1 Superoxide Dismuatse

SOD activity increased significantly in both genotypes under heat stress treatment at all stages of growth and highest SOD activity was recorded at 15 days after anthesis. C306 showed comparatively higher activity compared to PBW 343 at all stages of growth (Fig. 44). C 306 showed 13, 20 and 25% increase in SOD activity at vegetative stage, anthesis and 15 days after anthesis respectively, while, PBW 343 showed 4, 7 and 11% increase respectively.

4.4.1.9.2 Catalase

There was significant increase in catalase activity in C 306 at all stages of growth. The per cent of increase in catalase activity were 21, 24 and 24% under heat stress treatment compared to normal temperature at vegetative stage, anthesis and 15 days after anthesis respectively. However, maximum activity was recorded at 15 days after anthesis (Fig. 45). PBW 343 showed significant increase in catalase activity at vegetative stage only i.e., 8%, while during anthesis and 15 days after anthesis there was 23 and 11% reduction in catalase activity respectively. However, maximum catalase activity for C 306 was recorded at 15 days after anthesis in both treatments, while PBW 343 at normal temperature showed maximum activity at anthesis stage and under heat stress treatment maximum activity was recorded at vegetative stage.

4.4.1.9.3 Ascorbate Peroxidase

Increase in ascorbate peroxidase activity was clearly seen in both genotypes at all stages of growth when plants exposed to heat stress treatment and the value was highly significant in C 306 (Fig. 46). Highest activity in both genotypes was recorded at 15 days after anthesis. There was

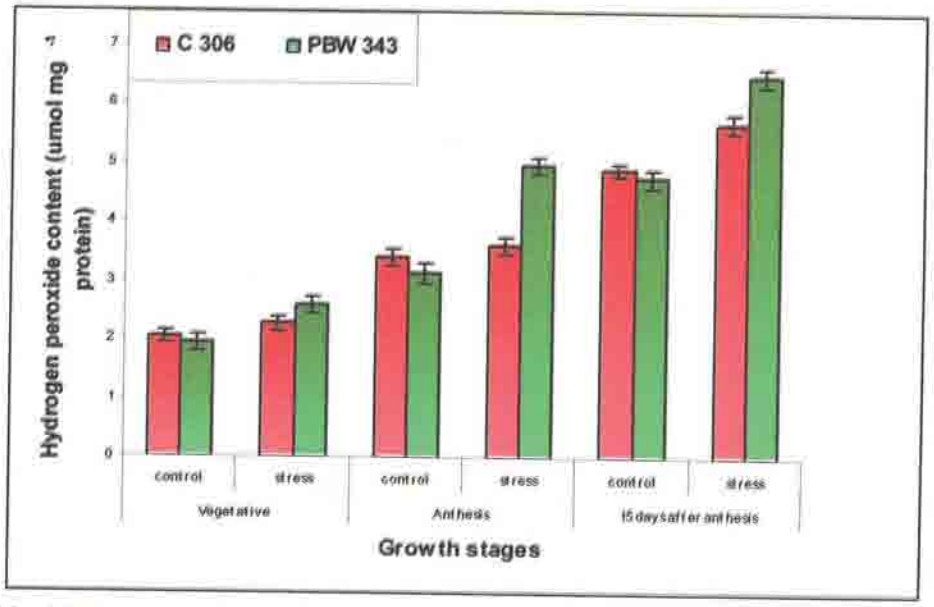

Fig. 43. Hydrogen peroxide content (µmol mg^{-1} protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

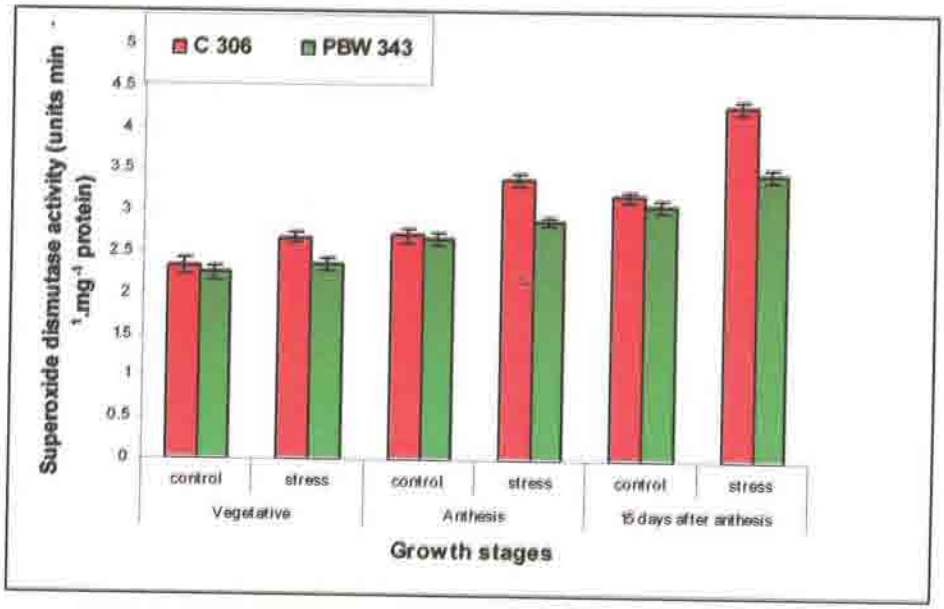

Fig. 44. Superoxide dismutase activity (units min^{-1} mg^{-1} protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

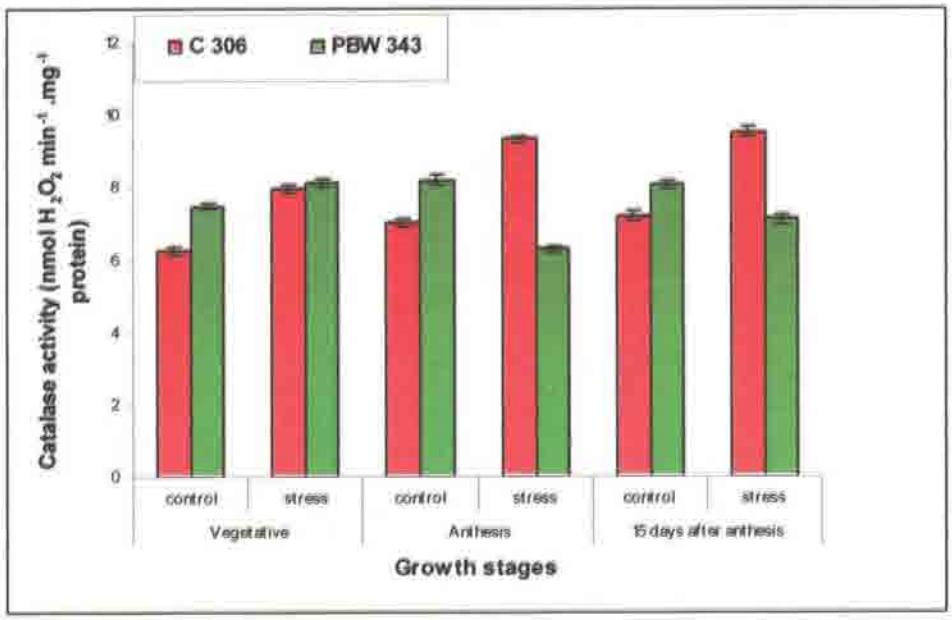

Fig. 45. Catalase activity (nmol H_2O_2 min^{-1} mg^{-1} protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

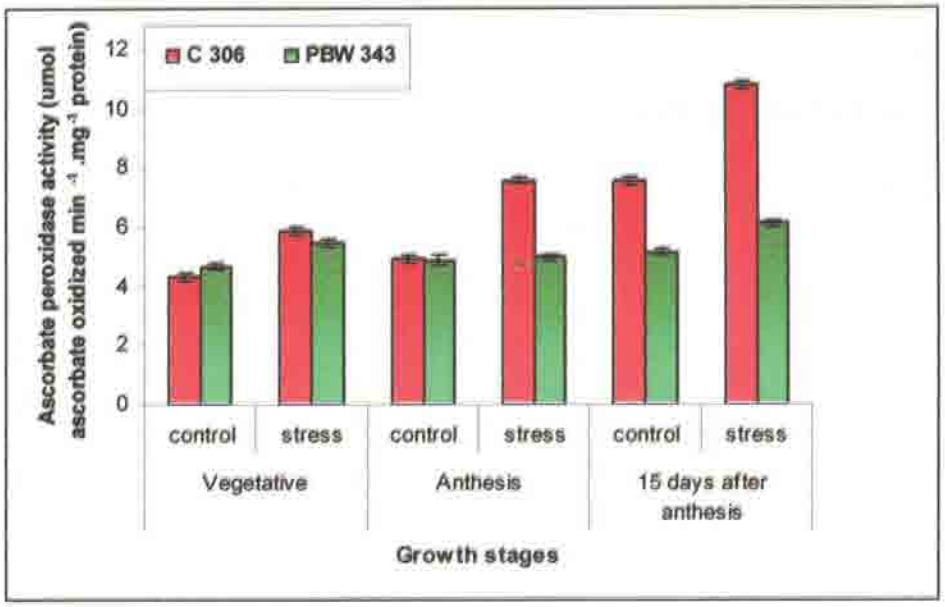

Fig. 46. Ascorbate peroxidase activity (µmol Ascorbate oxidized min^{-1} mg^{-1}protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

26, 35 and 30% increase in ascorbate peroxidase activity in C 306 at vegetative stage, anthesis and 15 days after anthesis respectively, while PBW 343 showed 14, 2 and 16% respectively increase in the enzyme activity under heat stress treatment compared to normal treatment.

4.4.1.9.4 Glutathione Reductase

Maximum GR activity in C 306 in both treatments was recorded at 15 days after anthesis. However, there was significant increase in the enzyme activity at all stages of growth in C 306 under heat stress treatment and there were 41, 35 and 37% increase in the enzyme activity under heat stress condition compared to normal temperature (Fig. 47). PBW 343 showed reduction in the enzyme activity under heat stress treatment compared to normal temperature at all stages of growth and maximum reduction in the enzyme activity was recorded at 15 days after anthesis. At all three growth stages there were 14, 3 and 23% reduction in the enzyme activity respectively.

4.4.1.9.5 Peroxidase

Significant increase in peroxidase activity was recorded at all stages of growth and maximum activity was observed at 15 days after anthesis. However, the per cent of increase in the enzyme activity under heat stress treatment was 21, 25 and 30% respectively in C 306. While PBW 343 showed maximum activity of the enzyme at 15 days after anthesis in both treatment and there was slight increase in the enzyme activity at vegetative stage i.e., 1%, while at anthesis and 15 days after anthesis there was 17 and 24% reduction in the enzyme activity respectively (Fig. 48).

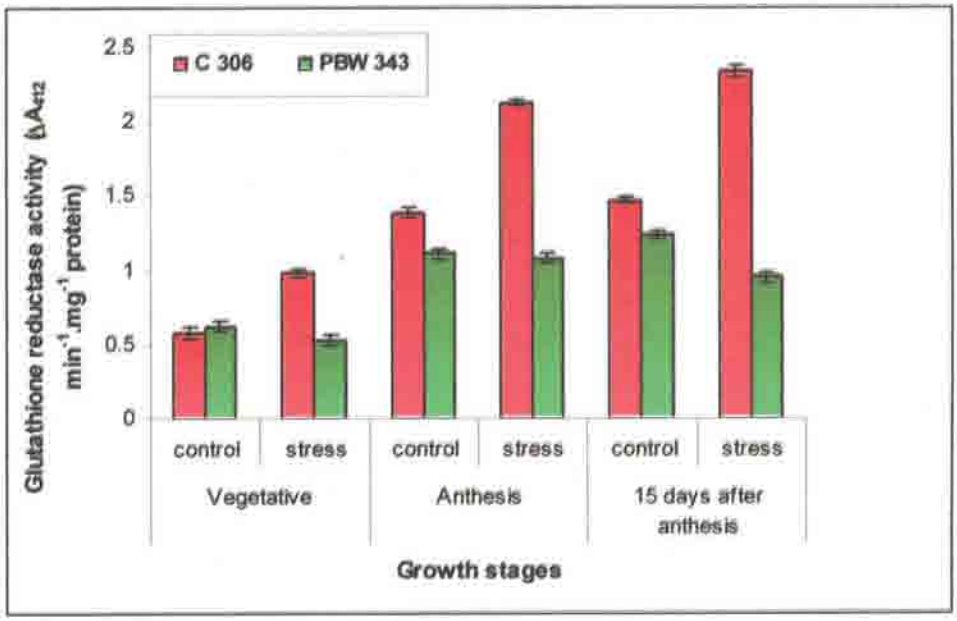

Fig. 47. Glutathione reductase activity (ΔA_{412} min^{-1} mg^{-1} protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

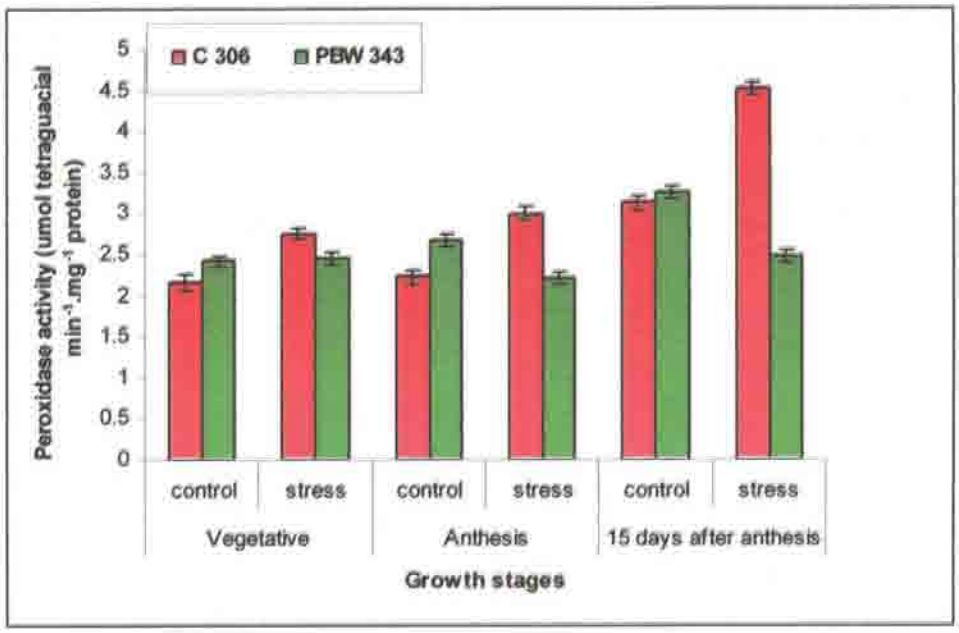

Fig. 48. Peroxidase activity (µmol tetraguacial min^{-1} mg^{-1}protein) in C306 and PBW 343 under normal temperature (25/18 ^{0}C) and high temperature (35/25 ^{0}C) at vegetative stage, anthesis and 15 days after anthesis.

4.4.2 Molecular Analysis of Heat Stress Response

Effect of heat stress on the expression of some key genes coding for proteins of the photosynthesis apparatus and involved in protection of plastid machinery against heat stress were investigated through RT-PCR (*rbcS* and *rbcL*) or northern analysis (D1 protein, *psbA*, *psbQ*, APX and HSP101).

4.4.2.1 Expression analysis of Rubisco Small (rbcS) and Large (rbcL) subunits

A single 480bp RT-PCR product of Rubisco small subunit was obtained in all the treatment. Heat susceptible genotype PBW 343 showed significant reduction in the expression of rbc(S) under heat stress treatment at all stages of growth namely, vegetative stage, anthesis and 15 days after anthesis. In contrast, in heat-tolerant genotype C 306 reduction in the expression of small subunit of Rubisco was recorded only at 15 days after anthesis (Fig. 49a). A single RT-PCR product of Rubisco large subunit of size 811 bp was obtained under heat stress and normal temperature treatment at all stages of growth. Expression of rbc(L) was not influenced by heat stress treatment in heat tolerant genotype C 306 at any of the growth stages. Whereas rbc(L) transcripts were less abundant in PBW 343 subjected to heat stress at anthesis and at 15 days after anthesis (Fig 49b).

4.4.2.2 Effect of heat stress on the expression of D1 protein (psbA)

RNA blot was probed with D1 gene sequence and a single transcript of size 689 nt was visualized in all treatments. At vegetative stage, a faint band was observed in C 306 under both normal and heat stress conditions whereas strong signals were detected in PBW 343. At anthesis stage expression of D1 gene was found to be similar in both the genotypes. However, C 306 showed

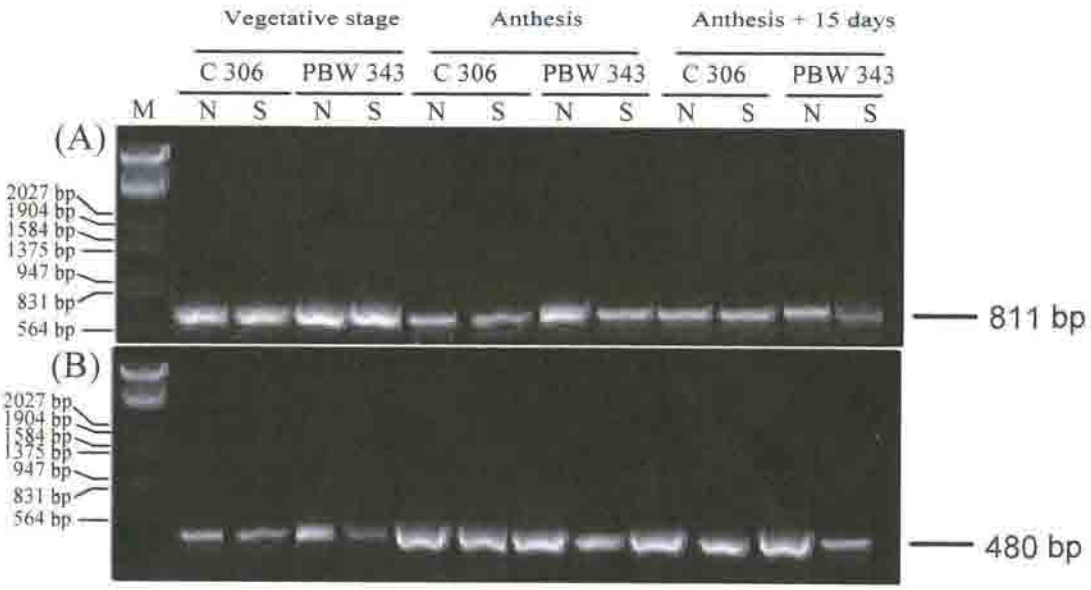

Fig. 49a&b Expression of genes encoding Rubisco large subunit (*rbcL*) and small subunit (*rbcS*) in response to high temperature stress in wheat genotypes differing in their heat tolerance. N, normal temperature (25/18°C day/night); S, high temperature (35/25°C day/night); Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature treated plant were collected at Vegetative stage (50 Days), anthesis and 15 days after anthesis; RNA was isolated and subjected to RT-PCR analysis; A) rbcL; B) rbcS. M = λ *Hin*d III/*Eco*RI marker.

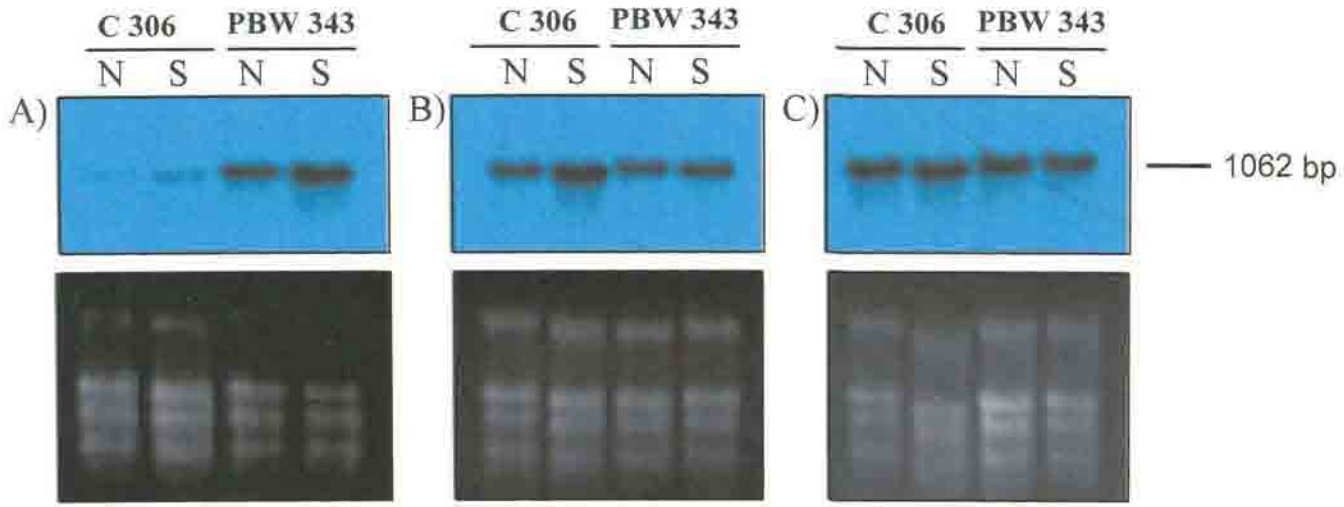

Figure 50. Expression of *psbA* gene encoding 32kDa protein of of photosystem II in response to high temperature stress in wheat genotypes differing in their heat tolerance. N, normal temperature (25/18°C day/night); S, high temperature (35/25°C day/night); Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature treated plant were collected at A) Vegetative stage (50 Days); B) Anthesis and C) Anthesis+15 days; RNA was isolated and subjected to Northern hybridization analysis. Lower panel shows ethidium bromide stained rRNA bands of gel used to prepare RNA blot above.

elevated D1 gene expression upon heat stress at this stage. In contrast, at 15 days after anthesis, high level of D1 gene expression was observed under normal and high temperature conditions in both the genotypes (Fig. 50).

4.4.2.3 Expression analysis of oxygen evolving complex (psbO) gene:

Probing of RNA gel blot with psbO gene sequence revealed a single band of size 690 nt in all the treatments. The intensity of bands was comparable in all the treatments indicating that the transcript level of psbO gene was not affected by heat stress (Fig. 51).

4.4.2.4 Expression analysis of thylakoid ascorbate peroxidase (APX) gene

Expression of APX gene was studied using northern analysis. A single transcript of size 636 nt was observed in all treatments at all growth stages when northern blot was hybridized with APX gene sequence. In C 306 expression of APX gene was comparable under normal and heat stress treatments at all three stages. However, in PBW 343, at vegetative stage under normal condition APX gene expression was lower than C 306. Upon heat stress, APX gene activity appeared to increase in PBW 343 to the levels observed in C 306. At other two stages (anthesis and 15 days after anthesis) APX transcript levels were comparable in all the treatments (Fig. 52).

4.4.2.5 Expression analysis of heat shock protein (HSP101) gene

When northern blots prepared with RNA from leaf tissue were probed with HSP101 gene probe a single band of 613 nt size was observed in all treatments. Under normal conditions, a high level of expression of HSP101 was revealed in both the genotypes at all the stages. However, heat susceptible genotype PBW 343 showed consistent decrease in transcript

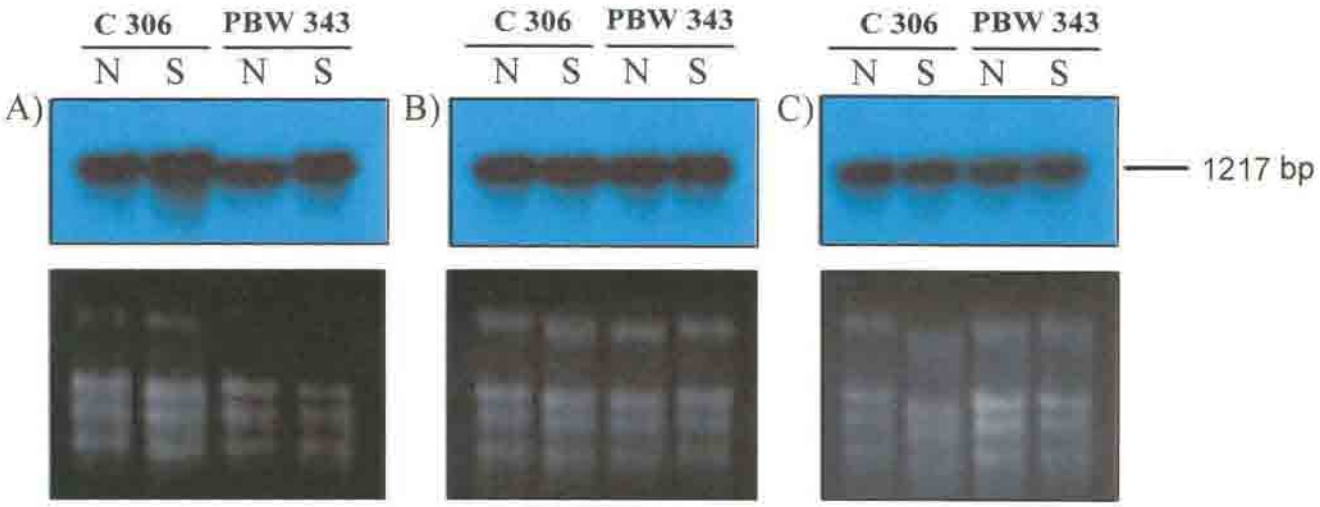

Figure 51. Expression of *psbO* gene encoding 33kDa protein of oxygen evolving complex of photosystem II in response to high temperature stress in wheat genotypes differing in their heat tolerance. N, normal temperature (25/18°C day/night); S, high temperature (35/25°C day/night); Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature treated plant were collected at A) Vegetative stage (50 Days); B) Anthesis and C) Anthesis+15 days; RNA was isolated and subjected to Northern hybridization analysis. Lower panel shows ethidium bromide stained rRNA bands of gel used to prepare RNA blot above.

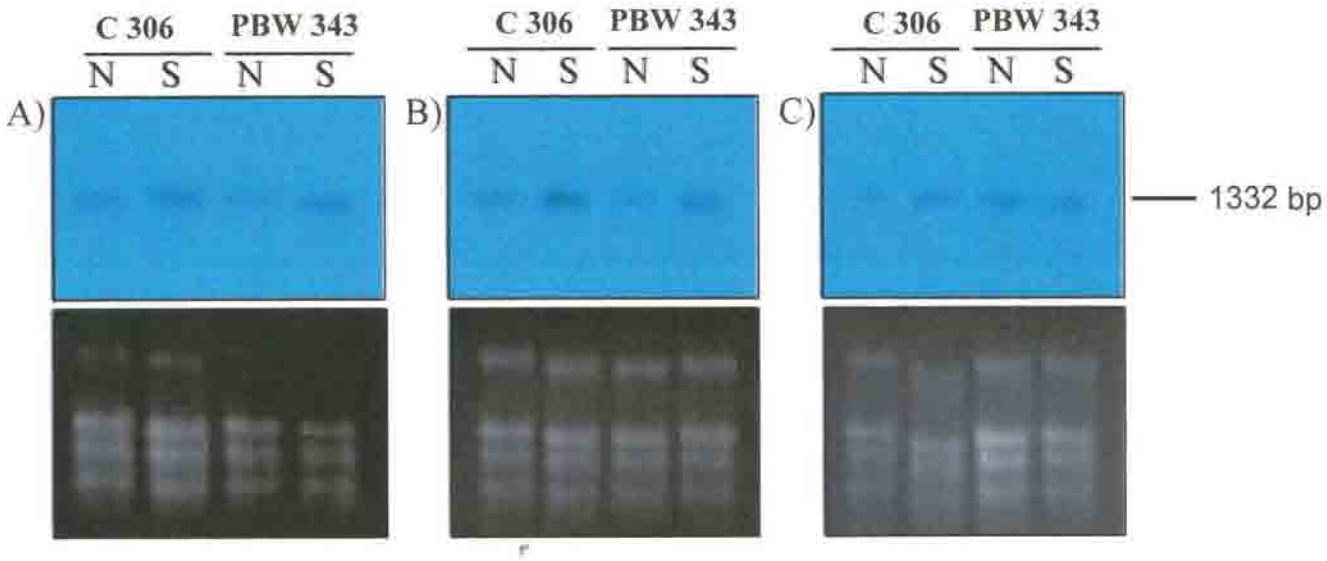

Fig. 52. *APX* expression in response to high temperature stress in wheat genotypes differing in their heat tolerance. N, normal temperature (25/18°C day/night); S, high temperature (35/25°C day/night); Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature treated plant were collected at A) Vegetative stage (50 Days); B) Anthesis and C) Anthesis+15 days; RNA was isolated and subjected to Northern hybridization analysis. Lower panel shows ethidium bromide stained rRNA bands of gel used to prepare RNA blot above.

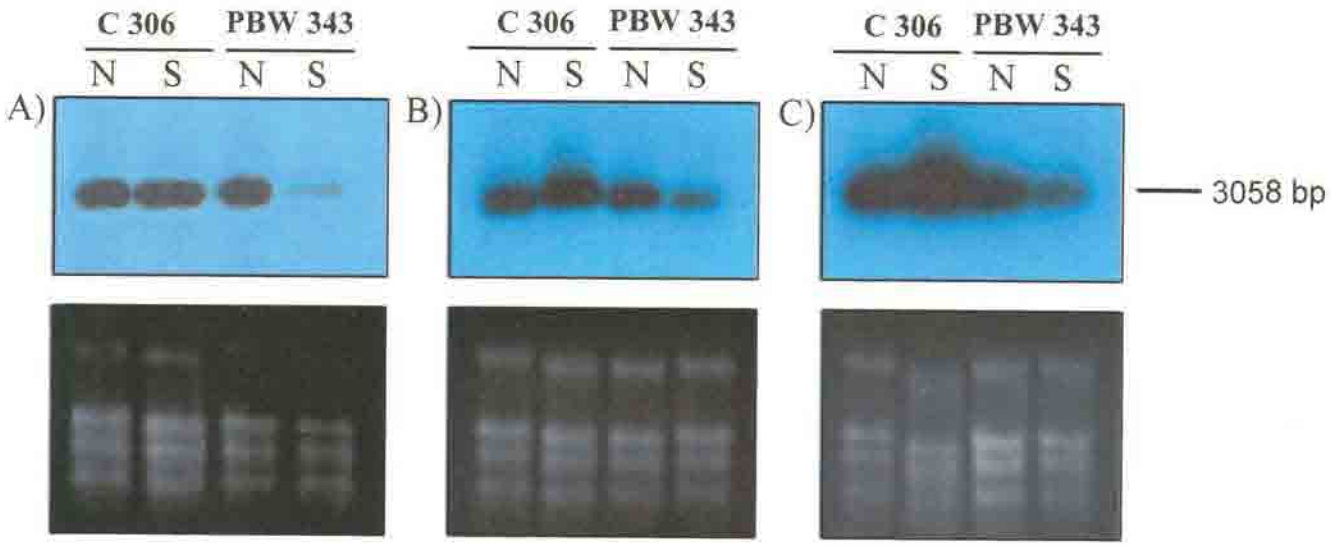

Fig. 53. *HSP101* expression in response to high temperature stress in wheat genotypes differing in their heat tolerance. N, normal temperature (25/18°C day/night); S, high temperature (35/25°C day/night); Plants were exposed to high temperature stress from 45 days after sowing till maturity. Leaf samples from control and high temperature treated plant were collected at A) Vegetative stage (50 Days); B) Anthesis and C) Anthesis+15 days; RNA was isolated and subjected to Northern hybridization analysis. Lower panel shows ethidium bromide stained rRNA bands of gel used to prepare RNA blot above.

abundance upon heat stress at all the three stages. In contrast, in heat tolerant genotype C 306 expression was increased upon heat stress and maximum expression was observed at anthesis and 15 days after anthesis stage (Fig 53).

5. DISCUSSION

5.1 EXPERIMENT-1

5.1.1 Physiological Parameters

The effect of delay in sowing was clearly seen through the significant reductions in all the physiological parameters, which could be explained on the basis of increase in temperature resulting in heat stress on wheat genotypes. Nagarajan and Rane (2002) reported that popularization of rice–wheat cropping system in north-western India has forced wheat to be sown late in about 2.5 million ha. This late sown crop gets exposed to mean maximum temperature of about 35 ^{0}C during grain growth period and causes yield reduction of 270 kg/ha/degree rise in temperature. There was significant reduction in chlorophyll content at all stages of growth under late planting condition compared to normal, and greater reductions were recorded in irrigated genotypes. Number of workers (Kaur, *et al.*, 1989, Ritchi *et al.*, 1990, Al-Katib and Paulsen 1990, Xu, 1991 and Amani *et al.*, 1996) has also reported reduction in chlorophyll content due to high temperature. Reynold *et al.* (1994) showed premature loss of chlorophyll due to heat sensitivity in wheat crop. There were significant differences amongst irrigated and rainfed genotypes in response to heat stress. Bhanu Prakash (1997), observed difference in leaf chlorophyll content in tolerant and susceptible wheat genotypes when subjected to moisture and high temperature stress.

There was significant reduction in MSI under late plantings compared to normal, and more reduction was recorded at reproductive stages compared to vegetative and this indicated increase susceptibility of the wheat crop to high temperature stress by advancing in age. Sullivan and Ross (1979), Raison *et al.* (1980) and Deshmukh *et al.* (1991) reported that stable cell membrane that remains functional under water/high temperature stress appears to control adaptation to both types of stresses. Christiansen, (1978) recorded that membrane disruption may alter water, ion and organic solute movement, photosynthesis and respiration.

Significant reduction in RWC was recorded at reproductive stages compared to vegetative stage under all plantings which indicated the increase in susceptibility of the genotypes to heat stress with age. The relatively less reduction in RWC and MSI in rainfed genotypes compared to irrigated genotypes under late planting condition may be due to their ability to adapt to water limited conditions. It was also clear that the genotypes which showed highest MSI under late plantings possessed high RWC. Deshmukh *et al.*, (1991) and Bhanu parkash, (1997) also observed that tolerant genotypes with relatively less membrane damage also possessed high RWC.

There was close association between transverse relaxation time T_2 and RWC and the genotypes, which maintained high RWC under late plantings also showed relatively high T_2 values. Irrigated genotypes showed more reduction in T_2 values under late plantings which might have resulted due to damage of cell membrane and structures under heat stress condition as was recorded by greater reduction in MSI in irrigated genotypes and the better adaptation by rainfed genotypes. Membranes are primarily composed of

proteins and lipids and any damages in these two components are likely to affect membrane stability. High temperature denatures membrane proteins and causes lipid phase transitions. Structural changes in the membranes are likely to affect the transverse relaxation time (T_2) of the cellular water (Nagarajan *et al.*, 2005). Maheswari *et al.* (1999) also reported that heat tolerant varieties of wheat, which suffered less membrane injury, had higher T_2 compared to susceptible varieties under heat stress condition, and the sharp decline in T_2 with increase in temperature corresponded to a loss of membrane integrity.

5.1.2 Growth Parameters

There was significant reduction in LAI, CGR, RGR, NAR and plant height under late and very late planting compared to normal and that may be because plants were subjected to high temperature stress under late plantings, which finally reflected in yield reductions. Saini (1988) reported that heat stress reduces the duration of vegetative growth and therefore reduces leaf area (Warrington *et al.*, 1977) and leaf number (Acevedo *et al.*, 1990). Rawson (1992) suggested that rate of leaf initiation will be faster at higher temperature. Takahashi and Nakaseko (1992) reported that delayed sowing decreased crop growth rate and net assimilation rate during the late grain filling period in wheat cultivars and decreased biomass. Under late plantings more reduction in all growth parameters was recorded at reproductive stages than vegetative stage and more reduction was observed in irrigated genotypes than rainfed genotypes. This is because the rainfed genotypes are adapted to high temperature.

5.1.3 Yield and Yield components

Significant reduction in yield and yield components were clearly seen under late and very late planting compared to normal and more reductions in all parameters was recorded in irrigated genotypes compared to rainfed genotypes under late plantings. Since high yield has been the primer objective of all research studies and in the present investigation the effort was made to understand the causes of low productivity under various plantings. The reduction in grain yield was due to reduction in both grain number per ear and 1000 grain weight. But the extent of reduction was more in 1000 grain weight in most of the genotypes. Many reports have indicated that unfavorable temperature exposures upon sowing early or late by deviating from recommended time reduced total plant biomass in wheat (Singh and Rajat, 1978, Randhawa *et al.*, 1981, Chaturvedi *et al.,* 1985 and Bhanu-Prakash, 1997). Wardlaw *et al.* (1989) reported that reduction in grain yield associated with high temperature following anthesis results from variation in kernel size and not due to changes in kernel number. Stone and Nicolas (1994) observed that short period of very high temperature (maximum >35 ^{0}C) in the post anthesis period can significantly reduce grain yield in wheat. Shah *et al.* (1994) and Abdelghani *et al.* (1994) reported that delayed sowing decreased 1000 grain weight. Owen, (1971) and Saini and Aspinall (1982) reported that reduction in grain number per ear due to high temperature at booting stage. Under hot conditions heat tolerant cultivars sustain relatively more kernel per spike than heat susceptible cultivars (Shpiler and Blum, 1991). Asana and Williams (1965) observed increase in grain weight was depressed beyond 25 ^{0}C due to increase in the respiration rate of grain. Bhullar and Jenner (1985)

indicated that high temperature imposed 10 days after anthesis reduced yield of wheat through effects on individual kernel mass. The significant reduction in yield under late plantings could be due to the fact that under high temperature conditions plant life cycle was shortened and the duration of all the phenophases were reduced, so that plant did not have enough assimilate for development of sink. It is interesting to note from the results of this study that close association were observed between physiological traits like chlorophyll content, MSI, RWC and T_2 and LAI, CGR and NAR which were responsible for biomass production. Reductions in chlorophyll content, MSI, RWC and LAI as a result of the heat stress resulted in reduction in photosynthetic rate which might have caused reductions in dry matter accumulation in plant tissues. High temperature reduces the yield drastically due to its detrimental effects on metabolism and duration of phenological phases (Warrington *et al.*, 1977, Saini and Dadhwal, 1986, Acevedo *et al.*, 1990, Jenner, 1991).

Sowing time significantly influenced the growth characters, yield and yield attributes of wheat. Plant height was significantly influenced and reduced as a result of delay in planting. Total dry matter accumulation per plant gradually increased with crop age and reached maximum at maturity. Dry matter accumulation decreased with delay in sowing from timely to very late. Late sowing resulted in lesser number of leaves, which lead to poor leaf area index coupled with lower net assimilation rate and CGR as well as dry matter accumulation. (Mishra *et al.*, 2003). Takahashi and Nakaseka (1992) reported that delayed sowing decreased crop growth rate and net assimilation rate during the late grain filling period in wheat cultivars and decreased biomass.

Blumenthal *et al.* (1990) and Sharma *et al.* (1991) have observed significant positive association of CGR with RGR, dry weight per ear and negative association of RGR with tiller per plant. The measurement of growth parameters CGR, RGR, LAI is important because they are closely related to biomass production. Their significance becomes crucial particularly under stress environment.

There was clear association between all physiological and growth parameters, yield and yield components under all plantings. The genotypes which showed less reduction in, chlorophyll content, MSI, RWC, relaxation time (T_2), LAI, CGR, RGR, NAR and plant height under late planting condition also showed less reduction in yield, eg., cultivars C 306, DL 153-2, HDR-77 and K 8027 (rainfed), HD 2815 and WR 544 (irrigated). Greater reduction in growth parameters, yield and yield components were recorded in HD 2781, RS 887 and HD 2866 (rainfed), HD 2329 and PBW 343 (irrigated). This association between physiological parameters, growth parameters and yield indicated that the reductions in chlorophyll content, MSI, RWC, relaxation time T_2, LAI, CGR. RGR and NAR under late and very late planting may be the reason for the significant decrease in yield. The short period upto flowering resulted in less vegetative growth such as height and leaf area in all the varieties (Rawson, 1992). High temperature reduces biological yield by reducing the effective leaf area through enhanced senescence and decreasing CO_2 assimilation (Kuroyanagi and Paulsen, 1985, Blum, 1986, Sayed *et al.*, 1989, Rawson, 1992). LAI was positively correlated with chlorophyll content and yield and negatively correlated with plant height (Kler and Bain, 1989). Reduction in leaf area will finally have negative effect on

yield since less leaves available for photosynthesis. Biomass accumulation is dependent on the crop growth duration, the photosynthesis rate and leaf area index and heat stress influence all these components (Blum, 1988).

It has been observed that the genotypes which showed high MSI under late planting conditions like C 306, HD 2815, also possessed higher chlorophyll content, RWC, T_2, LAI, CGR, RGR, NAR and plant height and maintained comparatively higher yield and were more stable under late plantings especially at reproductive stage. It is thus clear from the preceeding discussion that all physiological and growth parameters, yield and yield attributes in general are adversely affected by temperature stress. The impact of this adversity could be clearly seen to a large extent in genotypes recommended for irrigated conditions; however there were clear cut variation among the genotypes recommended for rainfed and late planting conditions.

5.2 EXPERIMENT 2 AND 3

5.2.1 Physiological and Biochemical Parameters

Data collected on the pot culture from five wheat genotypes showed reduction in chlorophyll content under late plantings, however more reduction in chlorophyll content was recorded at reproductive stages compared to vegetative stage in all the genotypes. Decrease in chlorophyll content due to high temperature stress has been reported by many workers. There was significant reduction in Pn rate under late plantings compared to normal as a result of exposure to heat stress, and greater reduction was recorded at reproductive stages compared to vegetative stage. Pastenes and Horton (1995) reported that increase in temperature from 20 to 25 ^{0}C increased photosynthesis with a Q_{10} value of 1.90. As the temperature increased from

25 to 30 ^{0}C the Q_{10} decreased possibly following a restriction in the regeneration of the acceptor for CO_2 and as temperature increased from 30 to 35 ^{0}C, Q_{10} was further decreased, as a result of the inability of the thylakoids to maintain adequate supply of NADPH. Physiological studies have shown that the rate of photosynthesis depends greatly on the temperature under which the plants are grown. Sharkova and Bubolo (1996) observed that high temperature reduced rate of potential photosynthesis. Further, increase in temperature results in premature plant senescence and reduced photosynthetic activity (Al-Katib and Paulsen, 1984). Al-Katib and Paulsen (1984) and Harding *et al.* (1990) reported that differences in photosynthesis among genotypes under high temperature stress are associated with a loss of chlorophyll and change in the chlorophyll a:b ratio due to premature leaf senescence.

Significant decrease in MSI was recorded under late and very late sown crop compared to normal. However, greater reduction in MSI was recorded at reproductive stages compared to vegetative stage. Blum (1988) reported that membrane is one of the sites of primary physiological injury by heat. Raison *et al.* (1980) reported that stable cell membrane that remains functional during stress appears to control adaptation to both heat and drought stresses. The reduction in MSI under heat stress condition could be the reason for the reduction in Pn rate under heat stress conditions. High temperature reduces the membrane stability and excessive fluidity of membrane lipids at high temperature is correlated with loss of physiological functions. The membrane disruption also causes inhibition of processes such

as photosynthesis that depend on the activity of membrane associated electron transport enzymes.

The rate of respiration increased significantly under late and very late planting compared to normal, due to higher prevailing temperature, particularly at reproductive stages. Evans *et al.* (1973) reported that dark respiration rate of wheat increases from 0.3 mg CO_2 dm^{-2} h^{-1} to 2.5 mg CO_2 dm^{-2} h^{-1} upon temperature up shift from 15 ^{0}C to 35 ^{0}C and thus contribute to considerable yield loss.

There was significant reduction in transverse relaxation time T_2 and longitudinal relaxation time (T_1) of leaf water under late plantings compared to normal and more reduction was recorded at reproductive stages. Longitudinal relaxation time (T_1) and transverse relaxation time (T_2) of barley leaves were found to be closely related to classical water status parameter like RWC. A linear relationship has been reported between RWC and leaf water T_1 value in wheat samples under water stress. This parameter has been extensively used to study water status, its compartmentation and variation under stress condition (Colire *et al.*, 1988). The significant reduction in T_2 and T_1 values under heat stress might be due to structural damage to the membrane during stress. The observations recorded on water potential under late plantings showed significant reduction compared to normal, and the genotypes which showed less reduction in chlorophyll content, MSI, Pn rate, T_2 and T_1 and lower RS rate under late planting condition possessed higher water potential. This might indicate the close association between all the physiological traits mentioned earlier and water potential.

Diverse environmental stresses differentially affect plant processes that lead to loss of cellular homeostasis accompanied by the formation of reactive oxygen species (ROS), which causes oxidative damage to membranes, lipids, proteins and nucleic acids (Srivalli *et al.*, 2003). The coordinate function of antioxidant enzymes such as SOD, catalase, APX and GR helps in processing of ROS and regeneration of ascorbate and glutathione redox metabolites (Halliwell, 1974, Wise, 1995, Asada, 1996, Foyer and Noctor, 2000). The oxidative damage to cellular components is limited under normal growing conditions due to efficient processing of ROS through a well coordinated and rapidly responsive antioxidant system consisting of several enzymes and redox metabolites. However, under various abiotic stresses the extent of ROS production exceeds the antioxidant defense capability of the cell resulting in cellular damages. It is clear from the data that there was a significant increase in the antioxidant enzymes activity in response to late and very late planting. Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzyme activity (Rui *et al.*, 1990, Gupta *et al.*, 1993, Badiani *et al.*, 1994, Zhau *et al.*, 1995, Sairam *et al.*, 2000). A considerable and significant increase in the activity of SOD under late planting and very late planting was recorded in all the genotypes. However, genotypes like HD 2815, HDR-77 showed more increase and higher SOD activity than other genotypes, which indicated that those two genotypes have better scavenging capacity and higher tolerance to heat stress than other genotypes. Many workers (Upadhyaya *et al.*, 1991, Gupta *et al.*, 1993, Jagtap and Bhargava, 1995, Davidson *et al.*, 1996) reported involvement of SOD in temperature stress tolerance. Catalase

activity increased significantly under late and very ate planting and tolerant genotype HD 2815 showed maximum increase at all growth stages. Upadhyaya *et al.* (1990), Olmos *et al.* (1994), Karus *et al.* (1995) reported that catalase is associated with the scavenging of H_2O_2 and an increase in its activity is related with increase in stress tolerance. Though there was reduction in the activity of GR at vegetative stage and at 15 days after anthesis, comparatively tolerant genotypes like HD 2815 and HDR-77 maintained relatively higher activity than other genotypes. Greater increase in APX activity at anthesis stage and at 15 days after anthesis in the late and very late planting in all genotypes showed induction of enzyme in response to increasing temperature and HD 2815 and HDR-77 showed higher activity under late planting, while under very late planting at anthesis stage HD 2815 and PBW 175 showed higher activity. Irina *et al.* (2002) reported that total cellular activity of APX appeared to be more sensitive to heat stress in plant compared to GR and SOD. Heat inducible transcriptional activation of cytosolic APX genes corresponds with an increase in APX activity (Bartosz, 1997, Foyer *et al.*, 1997). There was a significant decrease in peroxidase activity in late and very late sown crops. However tolerant genotype HD 2815 possessed highest value at 15 days after anthesis under the late and very late sowings:

5.2.2 Yield and Yield components

Significant reduction in yields and yield components was recorded under late sown condition. The reduction in yield as a result of heat stress may be due to the negative effect of high temperature on Pn rate and increase in RS rate thereby reducing the assimilate available for the sink

development. Moot *et al.* (1996) reported that for plants experiencing a 3 ^{0}C increase in day and night temperatures relative to local mean temperatures dry matter yields were reduced by 18% compared to control in wheat.

Yield reduction in wheat under stress can be caused by accelerated phasic development (Warrington *et al.*, 1977, Rawson and Bagga, 1979, Frank and Bauer, 1997), accelerated senescence (Kuroyanagi and Paulsen, 1985), increase in respiration (Berry and Bjorkman, 1980), reduction in photosynthesis (Blum, 1986, Conroy *et al.*, 1994) and inhibition of starch synthesis in developing kernels (Jenner, 1994). Singh *et al.* (1982) reported that photosynthetic rate and stomatal conductance were significantly correlated with the yield at all stages of crop development, but the relationship was found stronger during grain filling which might be due to premature loss of chlorophyll.. Wardlaw *et al.* (1989) reported that reduction in grain yield following post anthesis high temperature results from variation in kernel size and not due to changes in kernel number.

The results obtained in the present experiment also indicated that 1000 grain weight was more responsible for yield reductions than grain number per ear and tolerant genotypes showed less reduction in 1000 grain weight under late plantings compared to susceptible one. Shah *et al.* (1994) and Abdelghani *et al.* (1994) have also reported decrease in 1000 grain weight under late sowing. Ferris *et al.* (1998) reported that grain fertilization and grain set were most sensitive to high temperature condition. High temperature shorten plant life cycle and reduced the duration of all the phenophases which could be the reason for the significant reduction in yield under heat stress.

A perusal of the data shows that high temperature tolerant genotypes HD 2815 and HDR-77 showed increase in SOD, APX and CAT under late and very late sowing conditions and also maintained greater activity of GR and POX than susceptible genotypes like HD 2865, PBW 175, and PBW 343. These genotypes also showed less reduction in chlorophyll, MSI, Pn, T_2 and T_1 and less increase in Rs and maintained comparatively higher yields. This indicated the protective role played by antioxidant enzymes under heat stress and there significance in providing tolerance against heat stress condition.

5.3 EXPERIMENT 4

5.3.1 Physiological and Biochemical Parameters

From the data collected in the experiment which carried out in the phytotron with tolerant (C 306) and susceptible (PBW 343) genotypes significant and clear effect of heat stress in both wheat genotypes was observed and maximum reduction in chlorophyll content was recorded in PBW 343 compared to C 306. More reduction in photosynthesis rate due to high temperature treatment in PBW 343 could be explained by more reduction in chlorophyll content in this genotype. Investigation in soybean by Buttery *et al.* (1981) revealed that the variability in photosynthetic rate, nearly 44% was due to variability in chlorophyll content.

There was significant reduction in MSI in both genotypes under heat stress treatment at all stages of growth. More reduction in MSI was observed in PBW 343 compared to C 306 at all growth stages. Significant reduction in relaxation time T_1 was recorded in both genotypes at all stages of growth under heat stress treatment and greater reduction was recorded at 15 days after anthesis in PBW 343, while C 306 showed higher reduction at anthesis

stage. More reduction in T_1 value was recorded in PBW 343 under heat stress treatment at all growth stages. Water potential was significantly reduced in both genotypes at all stages under heat stress condition and more reduction was recorded in PBW 343. Reduction in water potential under high temperature stress in PBW 343 might cause structural damages to the membrane resulting in low T_d value. Rubisco activity decreased significantly under heat stress condition in both genotypes, and more reduction was recorded in PBW 343 at all growth stages, which could be the reason for the significant reduction in its Pn rate. Studies with cotton, wheat, tobacco, and maize have confirmed earlier observations (Weis, 1981a, 1981b, Kobza and Edwards, 1987) that Rubisco deactivates markedly in response to moderate heat stress (Law and Crafts-Brandner, 1999, Crafts-Brandner and Law, 2000, Crafts-Brandner and Salvucci, 2000, 2002). The reduction in photosynthesis rate could be explained by deactivation of Rubisco and change status of Rubisco from carboxylation to oxygenation. Evidences are available that inactivation of Rubisco is an early event in the inhibition of photosynthesis by elevated temperature (Weis, 1981a, 1981b, Kobza and Edwards, 1987). It has been further suggested that loss in activity of Rubisco activase during heat stress is caused by an exceptional sensitivity of the protein to thermal denaturation and is responsible, in part, for deactivation of Rubisco (Michael *et al.*, 2001).

Hydrogen peroxide content was significantly increased in both genotypes at all stages under heat stress treatment, and greater increase was recorded in PBW 343 at all growth stages. Smirnoff and Colombe (1988) suggested that the decrease in the capacity of the hydrogen peroxide

scavenging system may indicate the enhanced rate of hydrogen peroxide formation. Low CAT and SOD activities could be attributed to photoinactivation (Polle, 1997, Feierabend and Engel, 1986), which favors the accumulation of active oxygen species and causes damage to cell membranes (Dhindsa *et al.*, 1981).

Crop plants experience various stresses during their life cycle, such as high or low temperature, drought and salinity, which result in the formation of various reactive oxygen species (Sairam *et al.*, 2000). These reactive species can cause oxidative damage within the chloroplast, and also form highly reactive species, like $OH^{\cdot}$ (Asada 1996). Plants have developed enzymatic and non-enzymatic scavenging systems to quench active oxygen species. When plants are subjected to stresses such as high temperatures, the scavenging system may lose its function and the balance between producing and quenching of ROS can be disturbed, resulting in oxidative damage (Price *et al.*, 1989, Bowler *et al.*, 1992, Zhang and Kirkham, 1994). Under unfavorable condition an excess energy that has not been used for photosynthesis can produce the large amount of ROS and their products that induce damage to cell structure (Singh and Singhal, 2001).

There was significant increase in SOD and APX activities at all stages of growth under heat stress treatment. SOD activity which is responsible for scavenging $O_2^{\cdot}$ to produce H_2O_2, increased transiently at 12 d of drought, heat, or the combined stresses (Smirnoff, 1993). This increase in activity may reflect the enhanced amount of $O_2^{\cdot}$ production and also indicate the possible role for SOD's dismutation effects on $O_2^{\cdot}$ and protection of the photosynthetic

apparatus (Foster and Hess, 1982). The H_2O_2 scavenging enzyme, APX can remove H_2O_2 efficiently, especially in the chloroplast where CAT is absent (Groden and Beck, 1979). C 306 showed increase in the activity of catalase, GR and POX, while, PBW 343 showed significant reduction in these enzymes especially at reproductive stages (anthesis and 15 DAA) and this reduction in the activity could be the reason for the high susceptibility of PBW 343 to heat stress. Catalase, mostly localized in mitochondria and peroxisomes, breaks down and detoxifies H_2O_2. Reports on the effects of stresses on CAT activities varies, increased, decreased, or unchanged CAT activities under drought stress have been observed (Quartacci and Navari-Izzo, 1992, Smirnoff, 1993, Zhang and Kirkham, 1994 and Castillo, 1996). A reduction in CAT activity has occurred during short-time heat shock (Willekens *et al.*, 1995, Foyer *et al.*, 1997 and Dat *et al.*, 1998). The increased GR could protect the chloroplastic component against oxidation by H_2O_2 and minimize potential inactivation of SOD within chloroplasts (Foster and Hess, 1980). Peroxidation of membrane lipids has been observed at high temperatures (Mishra and Singhal, 1992; Upadhyaya *et al.,* 1990), which is a symptom of cellular injury. Enhanced synthesis of an anti-oxidant by plant tissues may increase cell tolerance to heat (Upadhyaya *et al.*, 1990,). Monk *et al.* (1989) reported that membrane lipid peroxidation occurred from the malfunction of the scavenging system, which could lead to damage to cellular components. Less MDA content accumulated under heat stress alone than during water and heat stresses, suggesting that less lipid peroxidation because of more stable SOD, higher CAT, APX and GR activities; and higher RWC were maintained in heat-stressed plants.

The significant increase in antioxidant enzymes system in C 306 at all stages of growth resulted in less membrane injury under heat stress and this was reflected in better water holding capacity like higher water potential and relaxation time T_1, chlorophyll content, Pn rate and Rubisco activity.

5.3.2 Molecular Analysis of Heat Stress Response

In the present study we employed RT-PCR and northern analysis to analyse changes in gene expression upon heat stress in two varieties of wheat with contrasting heat tolerance behavior. RT-PCR is regarded as less suitable for distinguish transcript abundance, especially when measurements are made based on ethidium bromide stain intensity after 30 cycles of PCR, nevertheless, we could detect differences in the expression of rbc(S) under heat stress, particularly in the heat susceptible genotype PBW 343.

The synthesis of RuBPcase/oxygenase, the most abundant protein on the earth is susceptible to heat stress. RuBPcase/oxygenase protein is composed of two subunits termed small (rbcS) and large (rbcL) which are coded by nuclear and plastid genome respectively. The small subunit synthesis is more heat susceptible than larger subunit in field grown soybean. There exist genetic differences even within a species for heat tolerance in RuBPcase/oxygenase SSU synthesis (Krishnan *et al.*, 1989). The data collected on Rubisco small and large subunits support the already existing reports and showed more reduction in small subunits under heat stress treatment particularly in susceptible genotypes PBW 343 especially at reproductive stages. At 34 ^{0}C, the heat tolerant wheat variety Mustang was able to maintain higher level of SSU synthesis than the heat susceptible variety Sturdy (Krishnan *et al.*, 1989). The sensitivity of SSU mRNA to heat

shock suggests that decreased synthesis of chloroplast proteins produced in the cytoplasm may be an important causal factor of heat damage to plants (Nover *et al.*, 1989).

PSII has been recognized as the most sensitive component of the complete photosynthetic system (Berry and Bjorkman 1980). The reaction center of PSII is considered to be the most sensitive component of the thylakoid membrane to thermal breakdown, and the function of the water-splitting D1 protein within PSII has been implicated as the most readily damaged by high temperature as well as being the primary target for photoinhibition (Aro *et al.*, 1993, Aro *et al.*, 1994). Chen *et al.*, (2004) found that high temperature and strong light stress induced a striking inactivation of PSII particle electron transport and degraded oxygen evolving complex 33 KDa polypeptide components.

The results obtained in this experiment showed that D1 protein and oxygen evolving complex has not been influenced by heat stress treatment in tolerant and susceptible genotypes at all the growth stages in spite of the pervious mentioned reports which showed that both components are highly sensitive to heat stress treatment.

Increased in the level of expression of ascorbate peroxidase at vegetative and anthesis stages was clearly seen in both genotypes under heat stress treatment and that might be the caused for the significant increased in the enzyme activity under stress condition. In contrast, at 15 days after anthesis there was no increased in the level of expression of ascorbate peroxidase and significant increased in the highest enzyme activity

were detected in both genotypes under heat stress treatment at that particular stage.

High degree of expression of HSP 101 at vegetative and reproductive stages was recorded in heat tolerant genotype C 306 under heat stress treatment, while susceptible genotype PBW 343 showed reduction in the expression of HSP 101 at all stages under stress treatment compared to control which could be the reason for the better performance of C 306 compared to PBW 343 under high temperature stress. The cells could not acquire thermotolerance, where HSP synthesis was blocked either at transcriptional (Johnston and Kucey, 1988) and/or translational level (Yamamori and Yura, 1982) or inactivated by antibodies (Riabowl *et al.*, 1988). Heat shock proteins (HSPs) are synthesized at high rate under high temperature stress and are thought to have a protective role. These are synthesized both under rapid heat stress or gradual increase in temperature. Three classes of proteins as distinguished by molecular weight account for most HSPs viz., HSP-90, HSP-70 and low molecular weight proteins of 15 to 30 KDa. HSPs act *in vitro* as molecular chaperones to bind partially denatured proteins, preventing irreversible protein inactivation and aggregation. The other compounds are also considered as heat shock proteins viz., 110 KDa polypeptides, ubiquities and GroEl proteins. Many heat shock proteins are also synthesized under heat stress and protect the nucleic acids and other cell components of plants (Rao, 2004).

Photosynthesis is one of the most important processes in plant system which is adversely affected by high temperature treatment (Bjorkman *et al.* 1974, 1975). It is well documented that high temperature inhibits

photosynthetic CO_2 fixation (Berry and Bjorkman, 1980). Al-Katib and Paulsen (1990) and Shah, (1992) reported that heat stress decreased mean photosynthetic rates by 32 and 11 % in seedlings and mature plants respectively.

The significant reduction in photosynthetic rate under heat stress treatments could be explained by the reduction in the expression of small and large subunits particularly at anthesis and post anthesis stages mainly in the heat susceptible genotype PBW 343 and more reduction in the expression of small subunit indicated that it's more susceptible and primary target of high temperature. The reduction in photosynthetic rate particularly in the susceptible genotype PBW 343 at all stages of growth may be explained by the significant effect of heat stress on gene coding Rubisco enzyme and not due to reduction in psbA and psbO under heat stress since no significant difference in the RNA transcripts was detected between control and heat stress treatment. Increase in the HSP101and thylakoid APX RNA transcripts under heat stress may have contributed to stress tolerance in C 306.

On the basis of the discussion covered so far it is realized that all the processes are interlinked together, molecular, biochemical, physiological and their final reflection in term of morphological traits. As seen the damage caused to the small subunit of Rubisco under heat stress could be the reason for the significant reduction in Pn rate. The reduction in water retention capacity of the plant under heat stress was associated reduction in MSI and all other physiological parameters and the damage has been shown to be minimized by better antioxidant enzymes activity particularly in tolerant genotype.

The existing variability in relation to various physiological traits and growth parameters is of immense importance for selection of parents which could be utilized in breeding programme for improvement of these traits which also show close association with biomass production. In general it will be a matter of further interest to study the inheritance of these traits particularly under adverse environment. This showed the importance of physiological traits and growth parameters and their use for classification of genotypes according to response to environmental factors.

SUMMARY AND CONCLUSION

Heat stress during post anthesis stage is considered a major constraint for yield in wheat and causes annually significant reduction in productivity of wheat. Current investigation was carried out in order to understand the physiological, biochemical and molecular mechanism of heat stress tolerance in wheat and to find a suitable and simple physiological traits which could be used for screening large number of wheat genotypes for heat stress tolerance and to identify tolerant wheat genotypes which could be used in breeding programme for improving heat stress tolerance.

Twenty wheat genotypes were grown in IARI field, twelve genotypes were rainfed viz., C 306, DL 153-2, HD 2781, HD 2865, HD 2866, HD 2868, HDR-77, HI 1500, RS 887, RS 888, K 8027 and PBW 175 and eight genotypes were irrigated viz., HD 2329, HD 2815, HD 2733, RS 827, RS 873, RS 854, PBW 343 and WR544. Sowing was done at three dates: normal (November), late (December) and very late (January) in order to expose them to different temperature regimes. Significant reductions in chlorophyll content, membrane stability index, relative water content, transverse relaxation time T_2, leaf area index, crop growth rate, relative growth rate, net assimilation rate, plant height, yield and yield components were recorded under late and very late planting in all genotypes. However, rainfed genotypes showed better performance and there was relatively less reduction in all parameters as compared to irrigated

genotypes and maintained relatively higher yield under stress conditions. Among the genotypes C 306, HDR-77, HD 2815 and WR 544 were superior in their performance and showed higher level of tolerance to heat stress. The other two experiments were conducted in the pot culture with five wheat genotypes viz., PBW 343, PBW 175, HD 2865, HDR-77 and HD 2815. Sowing was carried out at three dates as mentioned above.

Significant reductions in chlorophyll content, membrane stability index, transverse relaxation time (T_2), leaf spin lattice relaxation time (T_1), photosynthetic rate and water potential was recorded at all growth stages and accompanied by significant reduction in yield and increase in SOD, catalase, APX, POX and GR activity and increase in respiration rate was also recorded under late planting conditions. HDR-77 and HD 2815 maintained better physiological systems under stress conditions. Both genotypes possessed higher antioxidant enzyme activities and higher yield under high temperature.

Last experiment was conducted in the phytotron with two contrasting wheat genotypes C 306 and PBW 343. Temperature was maintained 25/18 ^{0}C (day/night) through out the experiment in one chamber (control), while temperature was raised to 35/25 ^{0}C (day/night) in the other chamber after maximum tillering (stress treatment). Significant reduction in all physiological traits (chlorophyll content, MSI, relaxation time T_1, photosynthesis, Rubisco enzyme activity and water potential) were recorded in both genotypes at all stages of growth under heat stress treatment compared to control and relatively more reduction was recorded in PBW 343. SOD, catalase, APX, POX and GR

activities increased significantly in C 306 under heat stress treatment and reduction in POX, APX and GR activity was recorded in PBW 343 under heat stress treatment compared to control particularly at reproductive stages.

The results of RT-PCR showed significant reduction in the expression of small subunit of Rubisco under heat stress treatment in PBW 343 at all stages of growth, while C 306 showed reduction in the expression of small subunit of Rubisco at post anthesis stage only. The expression of large subunit of Rubisco was not influenced by heat treatment in C 306 at all growth stages, on the contrary PBW 343 showed significant reduction in the expression under heat treatment during reproductive stages. There were no difference in the expression of D1 protein and oxygen evolving complex under heat stress compared to control in both genotypes. Increase in the expression of ascorbate peroxidase in both genotypes at vegetative and anthesis stage was recorded. Expression of HSP101 has been enhanced in C 306 at vegetative stage, anthesis and 15 days after anthesis, while decrease in the expression was recorded in PBW 343 at vegetative and anthesis stages.

The present investigation showed the significant effect of heat stress treatment on different physiological traits in genotypes like C 306, HD 2815, HDR-77 and WR 544 which could be used for improving heat tolerance in wheat to attain high productivity of wheat in the North-West zone of the country.

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