

**EFFECT OF HIGH TEMPERATURE AND
IRRADIANCE CAUSING FORCED SENESCENCE
IN WHEAT (*Triticum aestivum* L.) EXPRESSION
OF PHOTOSYNTHETIC GENES**

Thesis

SUBMITTED TO THE

G.B. Pant University of Agriculture & Technology
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By

Nidhi Srivastava

*IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF*

Doctor of Philosophy
(Plant Physiology)

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Dedicated to Him "The Omnipotent GOD"

...who gave me strength!

...who showed me various ways!

...who blessed me with nice people and ambience!

...To accomplish this task!!!

Dedicated to the "Gems" of my life....

....papa who inspires me to strive!

....mummy whose love and sacrifice made me to work!

....didi from whom I could know the real meaning of patience!

.....bhai and chotu who kept me alive with their sweet bantering !!!

Dr. Munna Singh
Assoc. Professor



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India

Certificate

This is to certify that the thesis entitled "**Effect of High Temperature and Irradiance Causing Forced Senescence in Wheat (*Triticum aestivum* L.) Expression of Photosynthetic Genes**" submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Plant Physiology** and minor in **Molecular Biology and Biotechnology** of the college of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bona fide research carried out by **Ms. Nidhi Srivastava, Id. No. 26620**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and source of literature have been duly acknowledged.

(Munna Singh)

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Advisory Committee

Certificate

We, the undersigned, members of the Advisory Committee of, **Ms. Nidhi Srivastava, Id. No. 26620**, a candidate for the degree of **Doctor of Philosophy** with major in **Plant Physiology** and minor in **Molecular Biology and Biotechnology**, agree that the thesis entitled "**Effect of High Temperature and Irradiance Causing Forced Senescence in Wheat (*Triticum aestivum* L.) : Expression of Photosynthetic Genes**" may be submitted in partial fulfillment of the requirements for the degree.



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(Anil Kumar Gaur)
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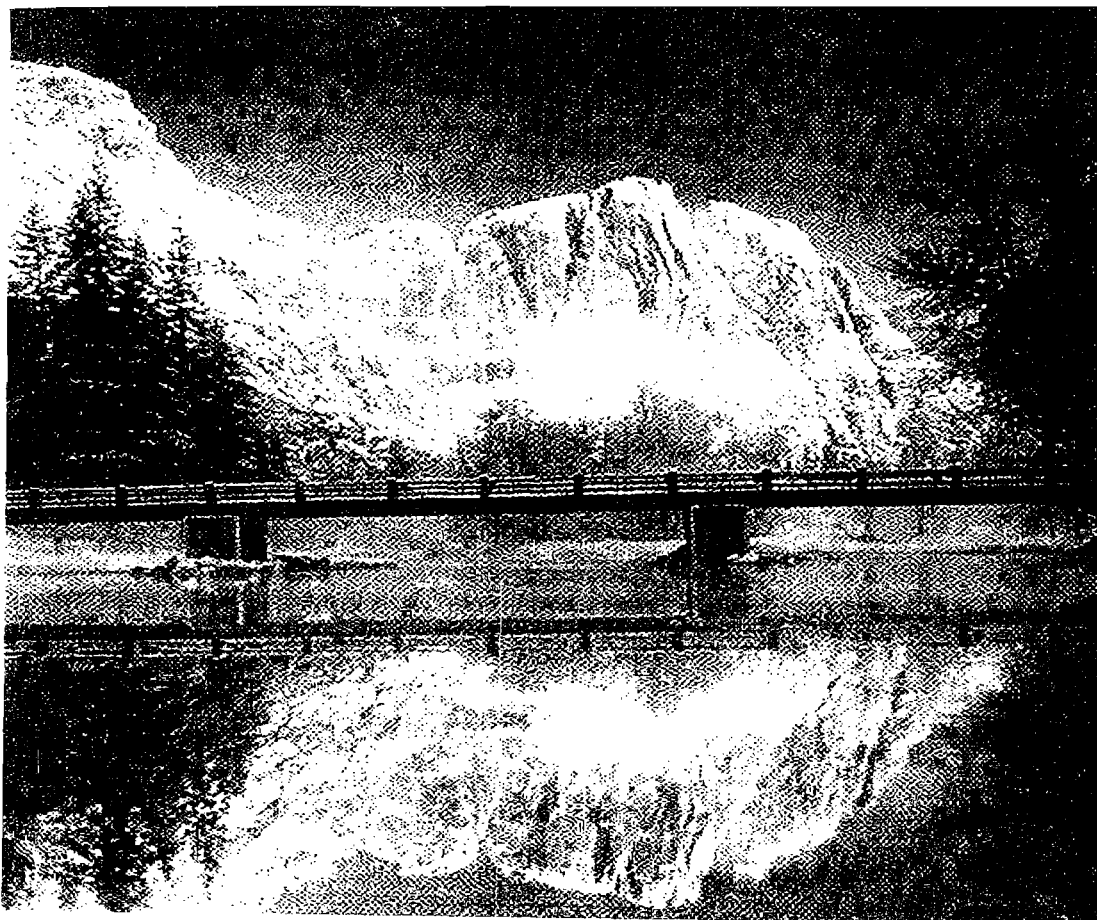
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I have lived long enough: my way of life
Is fall'n into the sear, the yellow leaf;

Macbeth Act V, Scene III



Introduction.....

Amongst cereals, wheat (*Triticum aestivum* L.) is the second most important crop after rice, and is a constituent of major cropping systems across the nation. The total wheat area in the world is about 226.945 million hectares producing about 609.556 million tonnes of grains with an average productivity of 2.686 tonnes/ ha. Wheat being the most important winter crop of India contributes 31.5% to the total food grain basket of the country. In India, wheat usually accounts for half the area under rabi food grain, particularly taking up field after field across UP, MP, Punjab, Rajasthan, Haryana and Bihar.

It has been envisaged that at least 110 million tonnes of wheat will be needed by 2020 A.D. (**Directorate of Wheat Research, 2000**). Since, very little scope exists for horizontal growth, the alternative seems by achieving vertical growth through increasing its productivity up to 35-40 q/ ha from the present level of 28.q/ha wheat production. There are several constraints which limits wheat production, and its productivity. Out of them, one of the major constraints is delayed sowing which ultimately results in exposure of plants to high temperature during anthesis / grain filling stage. The effective development of plant parts are pre- requisite for better expression of inherent potential and better utilization of environmental variables along with better acquisition of resources to develop , and also to produce the economic sink.

The genetic yield potential of a crop variety is limited by the environment, including abiotic and biotic stresses (**Oerke, 1999**). Plants are frequently exposed to these stresses under natural and agricultural conditions. Though the effects of biotic and abiotic stresses can be ameliorated up to certain extent, by the application of fertilizers, herbicides, pesticides and by irrigation etc. (**Cassells and Doyle, 2003**).

Amongst the abiotic stress, mainly strong light and low or elevated temperature, soil-salinity, waterlogging, global warming and drought may subject or adversely affect plant growth and performance (**Singh and Satoh, 2000; Santiago 2000; Yordanov et al., 2000; Else et al., 2001; Srivastava, 2001; Hortensteiner and Feller, 2002; Spano et al., 2003; Dalmia and Sawhney, 2004**).

Temperature is considered to be one of the most important environmental characteristics that drives the metabolic processes. (**Waston, 1999; Bosch and Penuelas, 2003; Farrant et al., 2004**). The anticipated rise in atmospheric temperature due to global warming effect of green house gases to the extent of 1-4.5°C over the present century can probably lead to substantial reduction in the growth and productivity of crop plants by changing their phenological and physiological processes such as spikelet sterility in rice (**Yoshida, 1981**), loss of pollen viability in sorghum, okra and maize (**Decker et al., 1986; Singh, 1997**) and reversal of vernalization and shortening the period of growth phases in wheat (**Abrol and Ingram, 1996; Aldesuquy, 2000; Lu et al., 2002, JIAO et al., 2003; Yang et al., 2004**).

In India, rice-wheat cropping system is the most widely adopted system. In the present scenario about 10.5 million hectare area is occupied by this cropping system, and it contributes about 25% of total food grain production in India. About 33% of India's rice and 42% of wheat is grown in this system. In most of the cases, rice-wheat system depends on the monsoon and thereby proper exploitation of varieties may not be possible. Due to late onset of monsoon, rice sowing may be delayed and that results in delayed sowing of wheat, which adversely affects its yield (**Bakshi et al., 2004**). Because, increased atmospheric temperature hastens the phenological development of the crop, causing forced senescence which impairs the grain filling periods along with lowering grain yield (**Smart, 1994; Singh et al., 2001; Spano et al., 2002; Munne-Bosch and Penuelas, 2003**). Inhibition of photosynthesis by heat stress

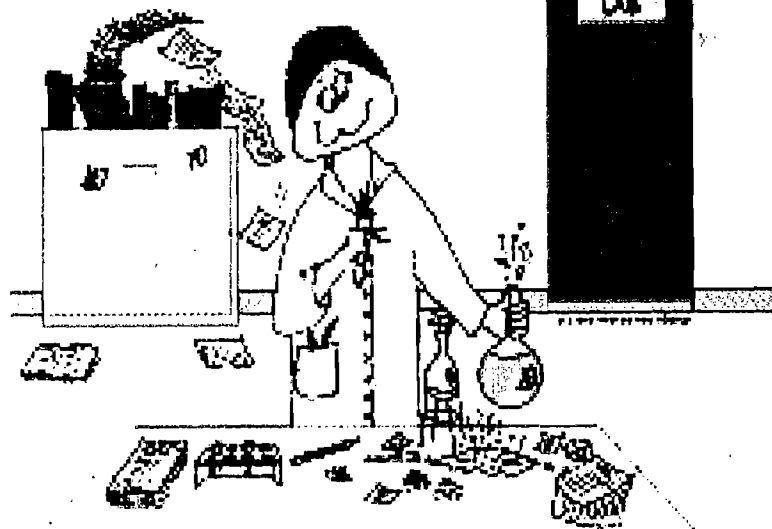
(30-40°C) is generally attributed to reduce rate of RUBP re-generation. The recent evidence shows that the inhibition of net photosynthesis correlates with a decrease in the activation states of Rubisco in both C₃ and C₄ plants which regulates the net photosynthesis (**Goyal, 2001; He et al., 2002; Jiao et al., 2003; Salvucci et al., 2004**). The early rise in temperature during March 2004, induced early and forced maturity without acquiring proper seed yield and seed quality (**Times of India, March; 2004**).

Genetic variation exists in the timing and rate of leaf senescence, both between species and genotypes. Furthermore, mutants also occur whose leaves remain green for longer than those of the parental genotypes. These are defined as 'stay-green' and examples of this phenotype have been identified in a number of crop species, including cereals (**Thomas and Smart, 1993; Spano et al., 2003**). To meet the demand for food from the growing world population, a significant increase in the world grain production is required, in developing countries.

Keeping in view the significance of genetic diversity for heat tolerance in wheat, field experiments were conducted with different wheat cultivars to study their growth and yield response and to find out heat insensitive genotypes for crop improvement, suitable under changing environmental variables. Consequently, following objectives were framed to reveal the suitability of wheat cultivars response to temperature as stated below:

1. To analyze consequences of high temperature and irradiance on plant performance/ photosynthetic efficiency and plant productivity.
2. Biochemical and ultra structural studies during forced senescence and senescence.
3. Differential behaviour of forced senescence affecting plant performance and protein products encoded by photosynthetic genes.

Dr. Jekyll



Hyde Laboratories

Review of literature.....

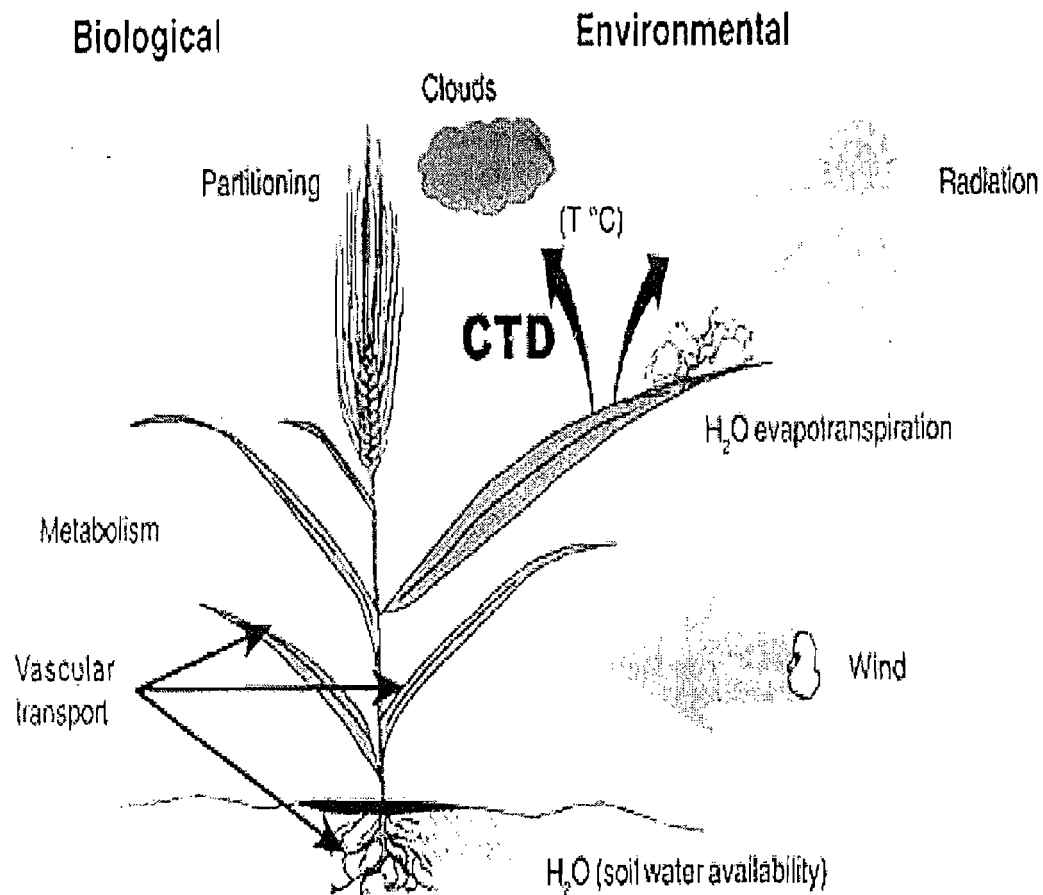


Fig 2.1 Plant under different environmental factors

Reynolds *et al.*, 2001b

Stress can be defined as an influence that is outside the normal range of homeostatic control in a given genotype (**Lerner, 1999a**). Plants frequently encounter stresses, both biotic (imposed by other organisms) or abiotic, arising from an excess/ deficit in the physical or chemical environment, limiting their genetic yield potential (**Oerke, 1999; Buchanan, 2003**). The effects of the biotic and abiotic stresses can be ameliorated by the application of herbicides, pesticides and by irrigation etc (**Cassells *et al.*, 2003**). Amongst the environmental conditions that cause damage to any plant/ crop are water logging, drought, high or low temperatures, excessive soil salinity, inadequate mineral nutrients in the soil, low or excess irradiance, toxic concentration of heavy metals, oxidative and mechanical stress (**Deskov *et al.*, 2000; Groot *et al.*, 2002; Cassells *et al.*, 2003; Schmitt, 2004; Mahan *et al.*, 2004**). Fig.(2.1)

Apart from all these abiotic stresses, there is also the emerging problem of global warming, expected to alter earth's climate globally under green house effect (**Alexandrov and Hoogenboom, 2000; Fumagalli *et al.*, 2001; Olesen and Bindi, 2001**). The abiotic as well as biotic factors may induce some common signals and responses in plants concomitantly and thereby have the potential to modulate the effect of each other through cross talking based on signal transduction pathways to produce reactive oxygen species as shown in Fig. (2.2) (**Amzallang, 2001; Schmitt, 2004**).

2.1 BIOTIC STRESS AND THEIR RESPONSES

Specific responses to biological stress involve the induction of antimicrobial proteins and phytoalexins (**Slusarenko *et al.*, 2000; Boller and Keen, 2000**). Upon stress perception, stress signal transduction takes place (**Bolwell, 1999; Ellis *et al.*, 2000; Nurnberger and Scheel, 2001**). Cross talk between the respective signaling systems with the suppression of one by the other leads to

Amongst the abiotic stress, mainly strong light and low or elevated temperature, soil-salinity, waterlogging, global warming and drought may subject or adversely affect plant growth and performance (**Singh and Satoh, 2000; Santiago 2000; Yordanov *et al.*, 2000; Else *et al.*, 2001; Srivastava, 2001; Hortensteiner and Feller, 2002; Spano *et al.*, 2003; Dalmia and Sawhney, 2004**).

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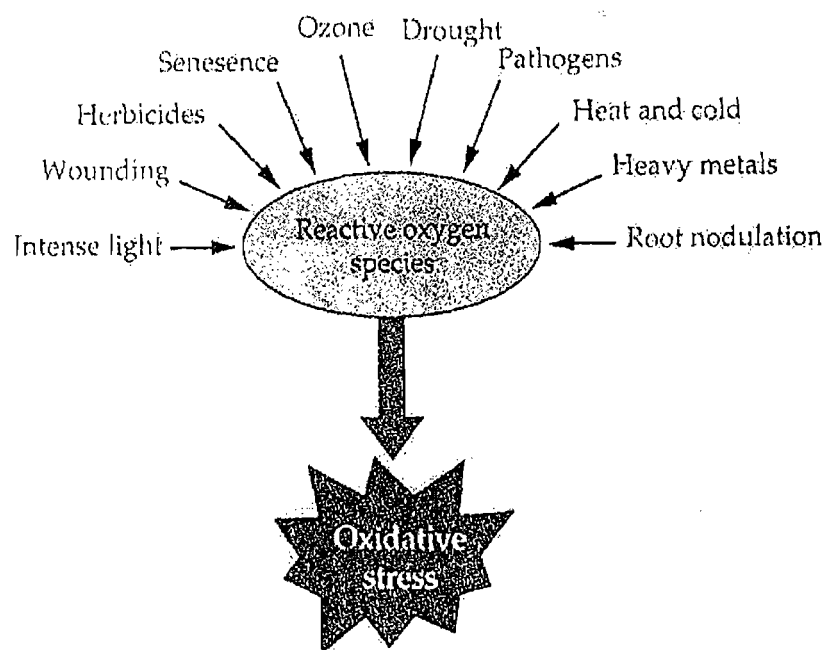


Fig. 2.2.: Environmental factors that induce the biosynthesis of reactive oxygen species (ROS) in plant cells (Buchanan *et al.*, 2004).

2.3 THE IMPACT OF THE ENVIRONMENTAL VARIABLES ON PHOTOSYNTHESIS AND PLANT PERFORMANCE

The effect of the environment on photosynthesis is of great interest to both plant physiologists and agronomists. From a physiological point, we wish to understand how photosynthesis responds to environmental factors such as light, ambient CO₂ concentration and temperature. The dependence of photosynthetic process is strongly associated with the environmental variables. It is equally important to agronomists because both plant productivity and crop yield, depend strongly on prevailing photosynthetic rates in a dynamic environment (**Taiz and Zeiger, 2003**). Plant rely on light as an essential energy source and signal for photosynthesis, biosynthesis of photosynthetic apparatus due to photomorphogenesis. The light requirements are largely variable in terms of intensities and spectral

distribution, depending on the plant species. The developmental stages of plants are also affected by photosynthetically active radiation (PAR). The seasonal and diurnal changes are also affected by the availability of light intensity (**Hideg et al., 2002**).

Measuring CO₂ fixation in intact leaves at increasing photon flux allows us to obtain a light response curves. In the dark there is no photosynthetic carbon assimilation and CO₂ is given off by the plant because of respiration and thereby CO₂ assimilation becomes negative. However, photosynthetic CO₂ assimilation increases upon illumination until it equates CO₂ release by mitochondrial respiration. The light intensity at which CO₂ uptake exactly balances CO₂ release is called the light compensation point. Increasing photon flux above the light compensation point results in a proportional increase in photosynthetic rate, yielding a linear relationship between photon flux and photosynthetic CO₂ assimilation indicates that photosynthesis is purely light regulated. Apart from this, upto certain extent photosynthesis needs continuous increase in the PAR, afterwards it becomes saturated. However, upon further increase in light intensity photoinhibition of photosynthesis may occur with the loss in the electron transport rate, rubisco activity, or the metabolisms of triose phosphates which impair photosynthetic performance and plant yield eventually (**Michael and Steven, 2004**).

After the light saturation point, photosynthesis is commonly referred to as CO₂ limited through the stomatal closure or mid-day depression, reflecting the inability of the Calvin cycle enzymes to keep pace with the absorbed light energy. The light response curve of most C₃ leaves saturates between 500- 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon fluxes, well below full sunlight, which is about 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (**Singh and Chaturvedi , 1997**). Because, upon excess absorption of irradiance , reaction center of PSII get photo-inactivated and the phenomenon is called as photoinhibition (**Singh et al ., 1997; Barth et al., 2001; Hideg et al., 2002; Lokhande et al., 2003; Singh, 2004**).

Under moderate excess light, dynamic photoinhibition (PI) is observed. Quantum efficiency decreases, but the maximum photosynthetic rate remains unchanged. This decrease is often temporary, and quantum efficiency can be recovered to its original value when photon flux decrease below saturation levels. However, chronic photoinhibition occurs normally at mid day, when leaves are exposed to maximum amounts of light coupled with elevated temperature and hot dry air (**Munne-Bosch, Penuelas, 2003**). This induces loss in C-fixation apparently along with damage to the photosynthetic membranes/ apparatus. Chronic photoinhibition (PI) is associated with irrecoverable damage of the D₁ protein associated with the reaction center of PSII (**Singh et al., 2005**) in contrast to dynamic PI. These effects are non-repairable and thus becomes fatal. (Fig 2. 4).

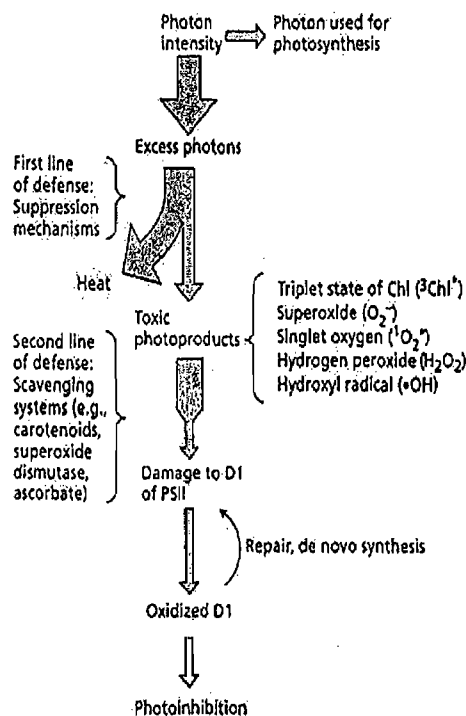


Fig. 2.4: Regulation of photo capture, photoprotection and repair of photodamaged photosynthetic system during light stress (Taiz and Zeiger, 2003).

2.3.1 Photosynthesis Vs CO₂

Photosynthesis is strongly limited at very low intercellular CO₂ concentrations, while respiratory rates are unaffected. As a result, there is negative balance between CO₂ fixed by photosynthesis and CO₂ produced by respiration. The CO₂ concentration at which these two processes balance each other known as the CO₂ compensation point, which regulates the net carbon economy of a plant. At low to intermediate CO₂ concentration, photosynthesis is limited by the carboxylation capacity of rubisco enzyme. However higher CO₂ levels also limit the capacity of Calvin cycle to regenerate the acceptor molecule i.e. ribulose-1, 5-bisphosphate, through the operation of photosynthetic electron transport system (**Michael and Steven, 2004**).

It should also be noted that C₃ plants may benefit more from on-going increase in atmospheric CO₂ concentration compared to C₄ plants. This happens due to differential carbon assimilating enzymes which initiates C- fixation in these plants. In C₃ plants, rubisco enzyme operates in both carboxylation and oxygenation modes depending upon the availability of CO₂ concentrations present in the environment. The CO₂ concentrating mechanism always promotes higher carbon fixation incase higher CO₂ are around the rubisco enzyme by suppressing its oxygenase mode of action. Due to lack of these characteristics C₄ plants show inability for better CO₂ sequestration strategy under higher levels of the CO₂ present in the environment. Thus, CO₂ sequestering mechanisms affect photosynthetic responses of leaves in C₃ plants significantly. Because of the operating CO₂ sequestering mechanisms in C₄ plants, CO₂ concentration at the carboxylation sites within C₄ chloroplasts is often saturating for rubisco activity. Consequently, plants with C₄-metabolisms perform dual CO₂ concentration and need less rubisco than C₃- plants for photosynthesis and also require less nitrogen to grow (**Goyal , 2001**). Fig (2.5)

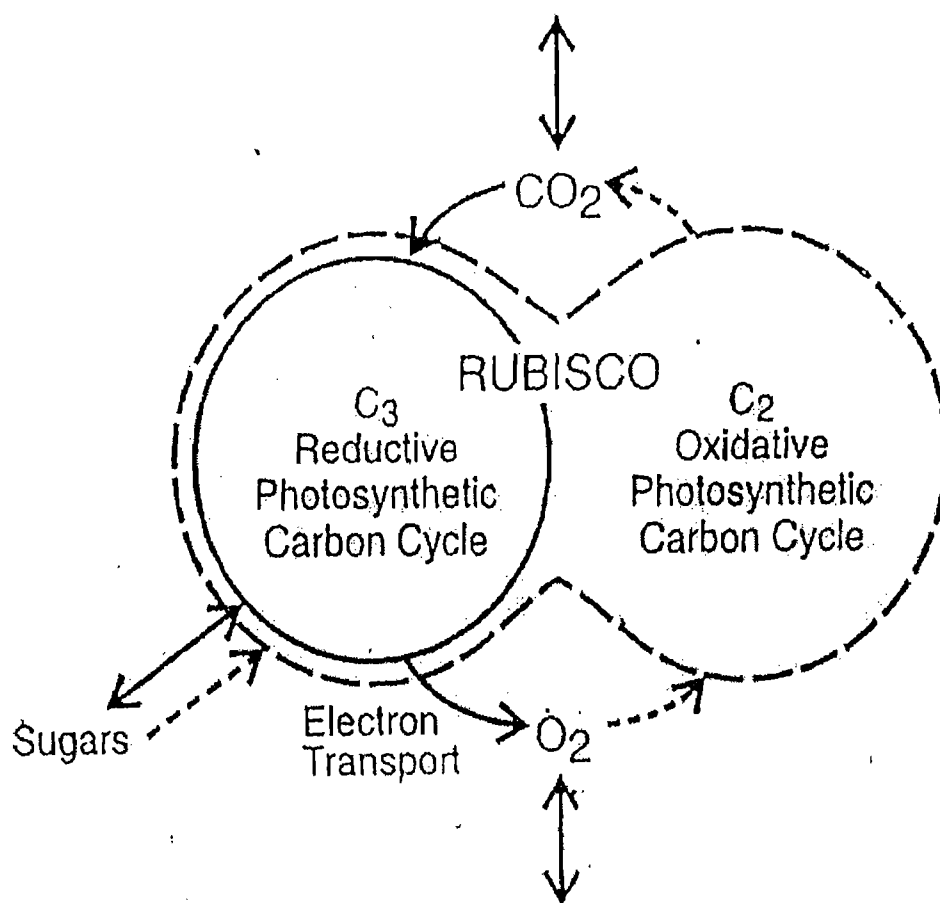


Fig. 2.5: The bifunctional activities of Rubisco enzyme: C₃ integrated operation of C₃ carbon assimilation cycle and C₂ photosynthetic carbon oxidative cycles regulated by CO₂ and O₂ availability (Goyal, 2001)

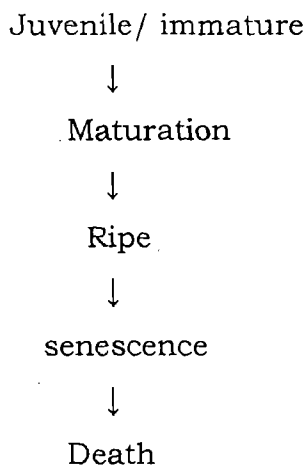
2.3.2 Photosynthetic response Vs temperature

Environmental temperature is a critical factor in the lives of almost all organisms. The change in temperature determines growth and development. Because, plants are suppose to experience thermal variation on diurnal and seasonal time scales, and are potentially more susceptible to thermal variation. Both, low and high temperatures can be lethal for plant functioning and its cellular components (Singh *et al.*, 1995; Behzadipour *et al.*, 1998; Mahan *et al.*, 2004).) At low temperature photosynthesis is often limited by phosphate availability to the chloroplast. The highest

photosynthetic rates are seen at optimal temperature. Incase, further rise in temperature occurs then photosynthetic rate starts decreasing. The exposure of photosynthetic organisms to temperature above the normal physiological range often results in an irreversible inactivation of photosynthesis (**Singh, 2000; Kimura *et al.*, 2002; Michael and Steven , 2004**).

2.4 SENESCENCE

Senescence represents the final stage of leaf development and is characterized by the transition from nutrient assimilation to nutrient remobilization (**Feller and Fischer, 1994; Hortensteiner and Feller, 2002**). The catabolic processes are well organized on the whole plant level (**Nooden, 1997**), on the organ level, on the cellular level (**Matile, 2000**), and on the subcellular level (**Matile, 1992**). Whole plant senescence in monocarpic plants such as wheat is the final stage of growth and development. It is an active, ordered process that involves remobilization of stored food from stems and leaves to the grains (**Gan and Amasine, 1997; Nooden *et al.*, 1997; Ori *et al.*, 1999**). However, ripeness acquires normal flavour, aroma, texture, juiciness and ready to eat. It occurs off or on the plant depending on the species. The senescence is the final stage of fruit development when fruits are beyond ripe followed by the decay. The acquisition of various stages in a plant's life can be visualized through following steps as stated below:



2.4.1 Types of senescence

Many annual plants, including major crop plants such as wheat, maize, and soybean after maturation show yellow symptoms of senescence to their seed production/ yield for the next generation or cropping system and also to fulfill agri- business requirements. The senescence of entire plant after a single reproductive cycle is called monocarpic senescence. Apart from these, other types of senescence may include the following:

- senescence of aerial shoots in herbaceous perennials
- seasonal leaf senescence in deciduous trees
- sequential leaf senescence in leaves after attaining the physiological maturity
- senescence of fleshy fruits and dry fruits.
- senescence of storage cotyledons and floral organs

The triggers of the various types of senescence are different. They can be either internal or external. Regardless of the initial stimulus, the different senescence patterns may share common internal programmes in which a regulatory senescence associated gene(s) eventually bring senescence followed by death.

2.4.2 Leaf senescence

Leaf senescence is a complex, highly regulated, developmental phase in the life of a plant that results in the coordinated degradation of macromolecules and the subsequent mobilization of components to other parts of the plant (**Buchanan and Wollaston, 1997, 2003**). It is a process that curtails leaf activity as a photo assimilate source and ends with leaf death (**Rousseaux et al., 2000**). Leaf senescence consists of set of degradative and remobilization activities that salvage valuable nutrients by reallocation to seeds or other viable parts of a plant. The senescence programme requires changes in gene expression and eventually culminates in death of the leaf or whole plant (**Chandlee, 2001**).

2.4.3 Why to study senescence?

As, this process is also of great practical value because during leaf senescence, nutrients are re-cycled to other parts of the plant. Nitrogen from leaves of deciduous trees is used for the synthesis of storage proteins (Clausen and Apel, 1991). However, in agriculture leaf senescence may limit yield in certain crops. It may also contributes to the post-harvest losses of vegetable crops (Gan and Amasino, 1997).

2.4.4 Environmental and autonomous regulation of leaf senescence

The limited water and nutrient availability (especially nitrogen) are major factors that adversely affect plants life. Plants have evolved mechanisms by which leaf senescence can be induced by these stresses to re-allocate nutrients to reproductive organs and to eliminate water consumption by older, less reproductive leaves. Thus, the regulation of leaf senescence has an obvious adaptive value, allowing the plant to complete its life cycle even under stressful conditions.

The leaf age has a major influence on the initiation of senescence. Leaves of many annual species exhibits a progressive decline in the rate of photosynthesis after full leaf expansion followed by senescence. (Hensel *et al.*, 1993; Chandlee , 2001). In wheat, senescence triggers results in the photosynthetic rate drops below a certain threshold. It may be near the CO₂ compensation point at which leaf no longer contributes carbon fixation to the rest of the plant. Longevity of leaves directly influence grain yield of wheat (*Triticum aestivum* L.), but is greatly diminished by environmental stress (Yang *et al.*, 2001). In most wheat growing regions and especially in the Mediterranean climate where temperature exceeds 35°C for several hours during a normal growing season, resulting induced senescence in the plants (Blum, 1996).

2.5 SENESCENCE MECHANISMS

Senescence and death are important process in the life cycle of an organism, that occur at many stages during the development and at many levels (**Nooden, 1988a**). Death may occur selectively in a few cells, in specific organs e.g. leaves or in a whole organisms. Senescence and death serve very diverse functions, and often, death is the end result of internally programmed senescence or degeneration process (**Thomas and Howarth, 2000**). The biochemical and molecular diagnosis of senescence associated mechanism can easily be explained with the help of Fig. (2.6), as shown below:

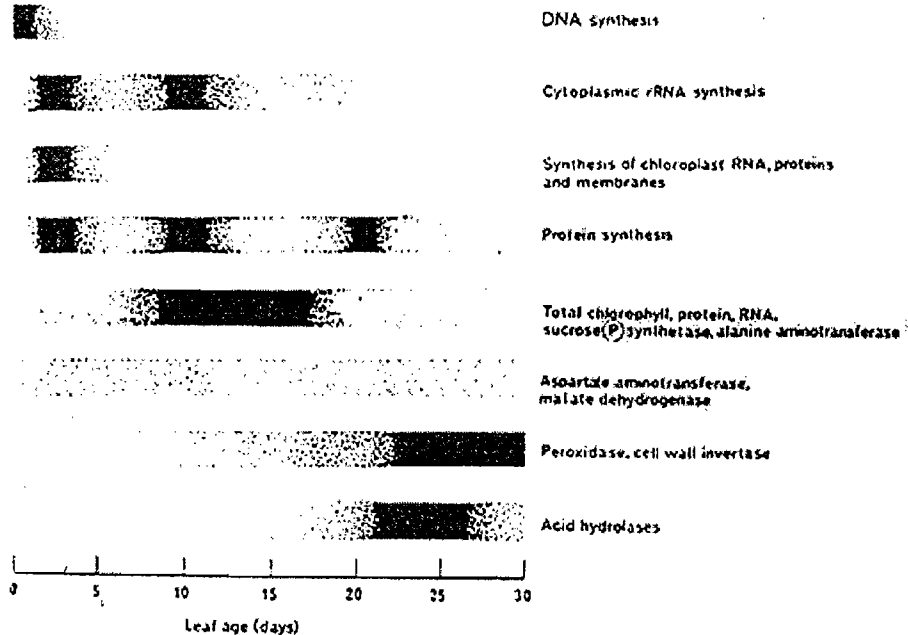


Fig. 2.6: Onset of molecular and metabolic events during development of a grass leaf from emergence to senescence. Density of shading indicates the level of the intensity for a specific activity (Stoddart and Thomas, 1998).

It is specific and orderly in terms of when, where and how it occurs (**Nooden and Leopold, 1978**). Senescence does progress with age. It is not simply a passive process, as it is controlled by internal and external signals. It can either be delayed or accelerated by altering senescence associated signals in contrast to aging where passive

accumulation of lesions takes place with age (**Smart, 1994; Nam, 1997**).

2.5.1 Programmed Cell Death

With regard to senescence, oxidative damage has been, a decisive cause to impair vital functions which eventually leads to death. This theory assumes that the gradual loss of capacity to detoxify reactive oxygen species results in progressive deteriorations of membranes, inducing membrane fluidity, degradation of phospholipids, lipid peroxidation and accumulation of products of peroxidative degradation of polyunsaturated fatty acids. It also includes changes in membrane proteins, and progressive leakage of ions and small molecules that finally lead to collapse the sub-cellular functions (**Thomson *et al.*, 1987**). Collectively, these observations suggest that death is a more or less coincidental event comparable to the crashing of a machine that had been kept in repair insufficiently. Although, the evidence is seemingly convincing, but the theory is unsatisfactory to explain the proper metabolic function in senescing cells (**Smart, 1994; Buchanan-Wollaston, 1997**). It seems to be justify to distinguish between metabolic leakage during senescence (export of solutes as associated with recycling of nutrients) and 'apoptotic leakage' due to collapse of solute compartmentation (**Okada 1995**). Hence, the program of senescence includes the terminal expression of genes encoding suicidal proteins.

2.5.2 Apoptosis Vs Senescence

Apoptosis has generally been applied to animal cells and tissues, while senescence has mostly referred to plant's organs and organisms. It often, but not always, shows some specific features i.e., chromatin condensation, building of the nucleus into small bodies. DNA fragmentation (called laddering due to its appearance on electrophoretic gels), 'boiling' of the cytoplasm, budding of the cytoplasm and lack of an inflammatory response in animals (**Arends and Wyllie, 1991**). Apoptosis can be initiated by wide range of factors

including disease, environmental stress, hormones and even metabolic imbalances (**Pennel and Lamb, 1997**). The final 'executioner' in apoptosis involves special thiol proteases, the ICE-like proteases that cleave many key enzymes and structural proteins internally (**Martin and Green, 1995**). The plants also have an apoptosis pathway similar to that found in animals. Typical apoptosis syndromes have been observed in plant cells undergoing the hypersensitive response viz., xylogenesis and multiple localized cell death patches (**Fukuda, 1996; Mittler and Lam, 1996**). However, the involvement of apoptosis associated with organ and organism senescence i.e., leaf and monocarpic senescence is uncertain.

2.5.3 Senescence Vs Nutrient Starvation

Senescence is closely associated with reproductive development and nutrients, often re-distributed from the vegetative parts to the developing reproductive sinks. The various forms of nutrient starvation have been involved during senescence of vegetative parts. (**Nooden, 1988c; Yang et al., 2001**). The nutrient-starvation theory states that the developing reproductive structures create a sugar deficiency in the shoot (**Sklensky and Davies, 1993**). The senescence is an active process, and during this process an adequate supply of carbohydrate is needed. It has also been proposed that the elevated sugar concentration may actually cause senescence. It has been known for a long time that photosynthesis is subject to a negative feedback regulation in the source-sink interplay and recent data indicate that sugar accumulation in leaves suppress the expression of some genes related to photosynthesis (**Gan and Amasino, 1997**). Therefore, the negative effect of sink removal from the photoassimilates has been termed 'functional senescence'. However, all negative metabolic adjustments are not listed under senescence at least in some species.

A broad syndrome of metabolic change occurs during senescence (**Nooden and Leopold, 1978**). It is difficult to determine which of these changes are central (primary) and peripheral

(secondary) during the on-set of the senescence process. Many of the enzymes that increase during senescence are clearly secondary for senescence. These enzymes metabolize the amino acids and lipids into transportable forms. In addition to this, some proteins such as metallothionein and peroxidase may be needed to protect the normal cell functions against the metal ions and peroxidase released (**Buchanan-Wallaston, 1997; Nam, 1997**).

2.5.4 Chlorophyll and Protein Catabolism Inside the Chloroplast:

Transition of Chloroplast into Gerontoplast

Physiological, biochemical and molecular changes of leaf cells undergo highly co-ordinated during senescence based on gene expression. The earliest and most significant change in cell structure is the breakdown of the chloroplast, the organelle that contains upto 70% of the leaf protein. Metabolically, carbon assimilation (photosynthesis) is replaced by catabolism of chlorophyll and macromolecules such as proteins, membrane lipids and RNA so that some of the released nutrients can be recycled. Several studies have shown that leaf cells undergo defined sub cellular changes during senescence (**Thomson and Platt-Aloia, 1987**). Primarily, the loss of chloroplast integrity occurs followed by the breakdown of the nucleus during senescence . Because, a large portion of the nitrogen in a leaf cell is located in the chloroplast (**Makino and Osmond, 1991**). It is expected that during leaf senescence chloroplasts are broken down along with other cellular constituents. However, nucleus remains intact for a longer duration to accomplish the recycling process successfully (Fig. 2.7).

In mesophyll cells, the population of chloroplast appears to be stable until very late stages of senescence (**Ford and Shibbes, 1988**). Chloroplast, persist and run through a senescence-specific differentiation. The final product of this transition is termed 'gerontoplast' (**Sitte et al., 1980**). The developing gerontoplasts are characterized by entirely catabolic metabolisms, unlike other types of plastids. The transition of chloroplast into gernatoplast is

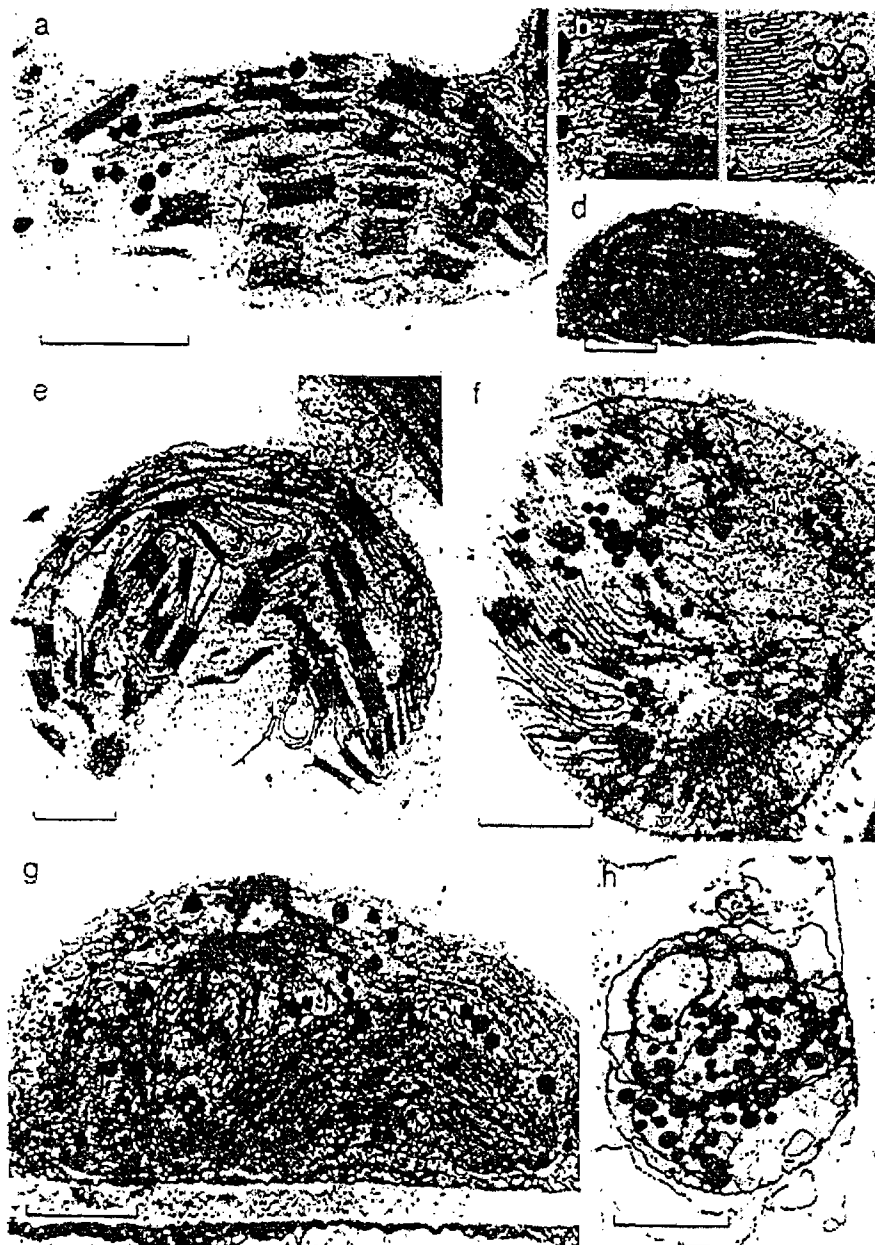


Fig. 2.7: Electron micrographs showing mesophyll chloroplast from *Festuca pratensis* during on-set of senescence. A: normal plastid day 0, b: plastoglobuli in normal plastid day 0, c: plastoglobuli in mutant day 0, d: normal plastid day 3, e: mutant plastid day 3, f: rapidly senescing, normal plastid day 3, g: mutant plastid day 6, h : normal plastid days 6 (Stoddart and Thomas, 1998).

characterized by structural changes in the thylakoids such as loosening and disorganization of grana stacks, swelling, vesiculation and progressive loss of membranes (**Matile, 1999; Thomas, 1997**). The stroma components also get lost along with decrease in their volume and density. At the end of the senescence period, fully differentiated gerontoplast consist of a still intact envelope wrapping up a large number of plastoglobules. Thus, developing gernatoplast seems to retain the internal organization that is required for reverse transition into Chloroplast.

The fate of thylakoid constituents and stromal enzymes are degraded early during senescence leading to the decline of photosynthetic capacity. However, the enzymes involved in carbon and nitrogen assimilation are lost and the amino acids derived from their catabolism may be exported via the phloem with or without prior modification (production of amides from other amino acids). Glutamine synthetase, the key enzyme in the assimilation of ammonia is present in the chloroplasts. It is susceptible to proteolysis in isolated chloroplast and gets degraded more rapidly than Rubisco or other enzymes involved in carbon assimilation (**Mitsuhashi and Feller, 1992; Thoenen and Feller, 1998**). The cytosolic form of glutamine synthetase was shown to be localized in the vascular bundles of rice leaves . Based on immunological data, a shift in ammonia assimilation from the chloroplast to the cytosol of mesophyll cells of tobacco has also been monitored .

It has been reported that stromal enzymes get degraded in isolated pea chloroplasts (**Mitsuhashi and Feller, 1992; Thoenen and Feller, 1998**). The degradation of the predominant stromal protein Rubisco in C₃-plants rubisco has been the subject of several studies (**Desimone et al., 1996; Ishida et al., 1998**). In intact chloroplasts, degradation of Rubisco causes the accumulation of different fragment patterns depending on the specific experimental conditions e.g., pH, energy status, solute concentration and free radicals . It has often been suggested that Rubisco degradation may

be initiated by reactive oxygen species (Desimone *et al.*, 1998; Ishida *et al.*, 1998; Roulin and Feller, 1998;) during senescence. A direct (non-enzymatic) cleavage of the large subunit of Rubisco into a 37 kDa and 16 kDa fragments induced by ROS has also been reported (Ishida *et al.*, 1998). The complete hydrolysis of proteins to free amino acids occurs due to the action of endo- and exopeptidases. It appears likely that the proteolytic system plays a role in chloroplast development to acquire housekeeping proteins. The Clp protease may also be important for the degradation of polypeptides in the cytochrome *f* complex. Hence, this proteolytic system is important mainly prior to the onset of senescence (Hortensteiner and Feller, 2002) (Fig. 2.8).

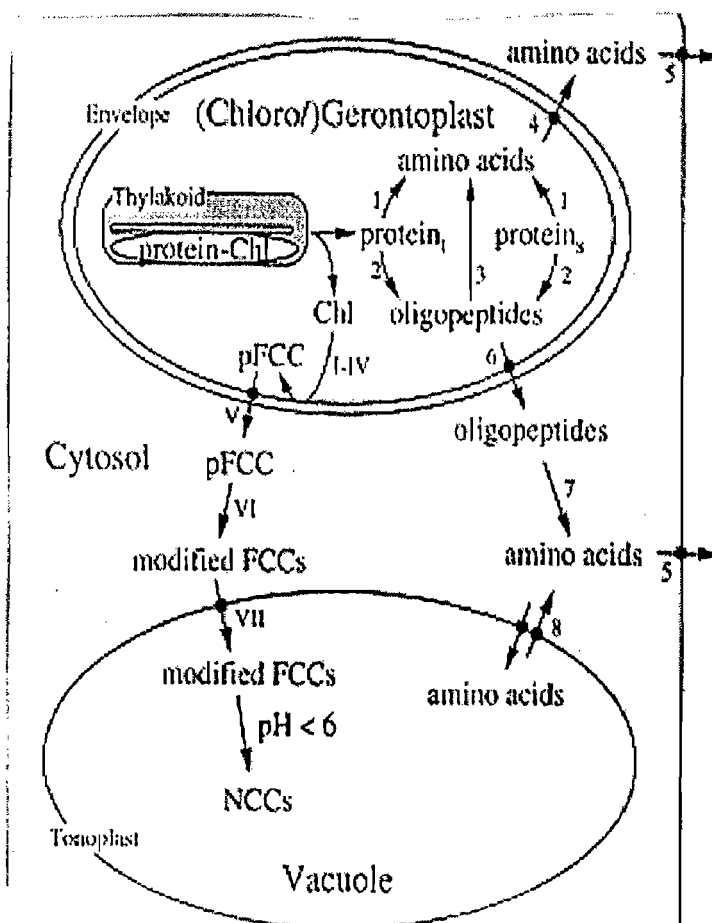


Fig. 2.8: The degradation pathway of Chl (roman numbers) and chloroplast protein and chloroplast proteins (Arabic numbers). Thylakoid and stromal proteins are represented by small t and s respectively (Hörtiensteiner and Feller, 2002)

I. chlorophyllase	1. exopeptidase
II. Mg dechelataase	2. endopeptidase
III. Pheide a oxygenase	3. exopeptidase
IV. RCC reductase	4. amino acid transporter (envelope)
V. Chl catabolite translocator	5. amino acid exporter (plasma membrane)
VI. FCC modifying enzymes	6. Oligopeptide transporter
VII. ABC transporter	7. cytosolic exopeptidases
	8. amino acid transporters (tonoplast)

2.5.5 Breakdown of Thylakoid-bound Proteins

The morphological analysis of senescence chloroplasts reveals dramatic changes of the thylakoid membrane system. The contact between grana stacks is loosened, and finally the membranes disappear in conjunction with an increase in the number and size of plastoglobuli. Disappearance of the membrane system occurs due to the degradation of membrane constituents such as lipids, proteins and chlorophyll (**Matile, 1992**). The nitrogen contents are most important in terms of the thylakoid-bound apoproteins of chlorophyll viz. LHCPII. Hence, in addition to Rubisco, degradation of LHCPII is also a major contributor to the overall loss of chloroplast proteins during foliar senescence. Therefore, the chlorophyll catabolism plays an important role for nutrient re-cycling in senescence. Because, catabolism of Chl during leaf senescence is accompanied by the loss of green color and is one of the most spectacular phenomenon in nature. The breakdown pathway has been nearly completely elucidated during the last decade, starting from 1991 with the first description of a Chl catabolite from barley (**Krautler et al., 1991**) to non-fluorescence Chl catabolites (NCCs) (**Muhlecker and Kraitler, 1996; Curty and Engel, 1996**), found in number of different species and are also believed to represent the final breakdown products of Chl that are deposited in the central vacuole. (Fig.2.9)

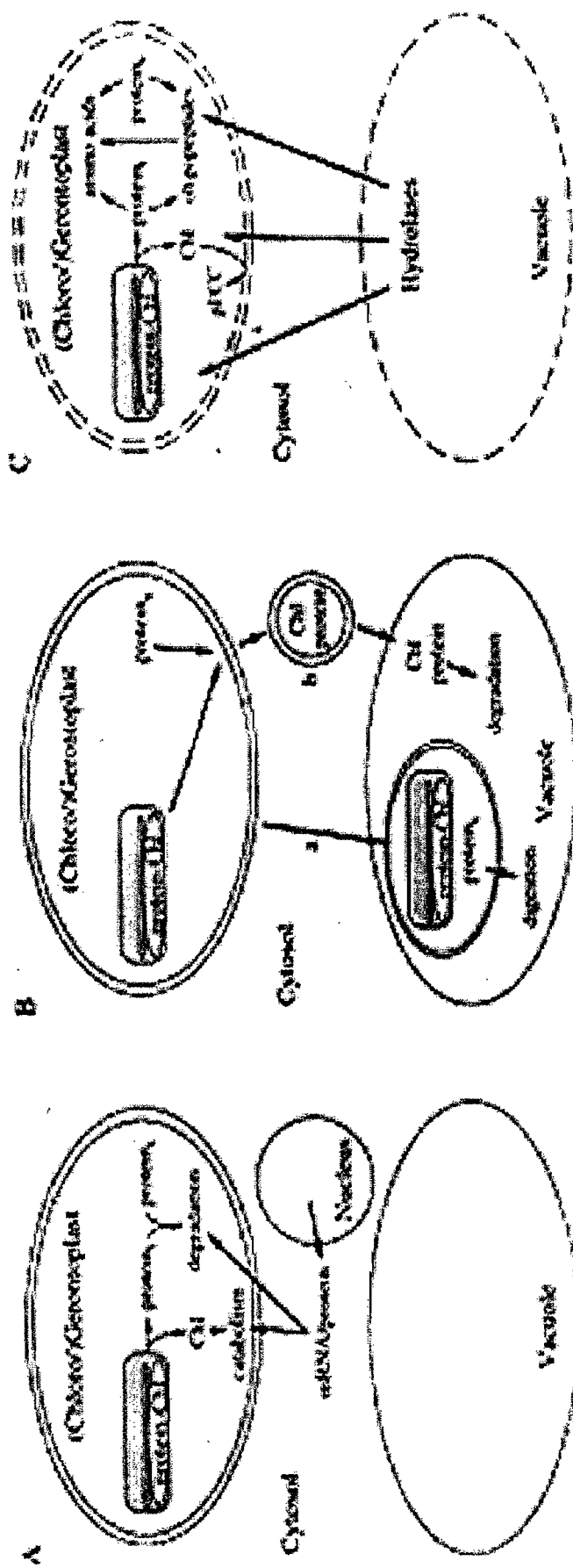


Fig. 2.9: Extraplastidial contributions to the degradation of chloroplast constituents (A) Involvement of nuclear encoded enzymes in the degradation of proteins and of chl inside the chloroplast. (B) Hypothetical model for the catabolism of chloroplast-derived components in the vacuole. (C) Degradation of chloroplast constituents by extraplastidial enzyme after loss of membrane intactness (Hörtiensteiner and Feller, 2002).

It has been investigated that the Chlorophyll is catalyzed by at least five enzymatic reactions commonly found in all species. The first four reactions take place in the senescence chloroplasts (gerontoplasts), starting with the removal of the phytol chain by chlorophyllase producing chlorophyllide (chlide). Chlorophyllase, is localized in the inner envelope membrane (**Matile *et al.*, 1999**). Chlorophyllase genes have been recently identified from *Arabidopsis*, *Citrus* and *Chenopodium* (**Jakob-Wilk *et al.*, 1999**; **Tsuchiya *et al.*, 1999**). Mg-free forms of chlorophyll have been identified in several plant species during senescence (**Ziegler *et al.*, 1998**). Thus, the removal of the central atom Mg of Chlide by Mg dechelatase to yield Pheophorbide was postulated to represent the second enzyme in the Chlorophyll catabolic pathway. It appears to be associated with a heat stable substance of low molecular weight (**Shioi *et al.*, 1996**).

The third reaction in the Chlorophyll catabolic pathways is most important for leaf yellowing, because it is responsible for the loss of green colour. Actually, two enzymes are necessary for the formation of the first identifiable cleavage product of Pheophorbide a, a primary fluorescent Chlorophyll catabolite (pFCC). However, Pheide a oxygenase (PaO) opens the porphyrin macrocycle by the introduction

of oxygen. This yields a red Chl catabolite (RCC) intermediate that is released from PaO after a site-specific reduction catalyzed by RCC reductase (**Rodoni et al., 1998**). The RCC activity has been tested in roots, and is measurable in the stroma at all stages of leaf development (**Rodoni et al., 1998; Takamiya et al., 2000**). However, PaO is bound to the inner envelope membrane and its activity is only detectable during senescence (Fig.2.10).

Metal chelation and reconstitution experiments have shown that PaO is a non-haem iron containing mono-oxygenase (**Hortensteiner, 1999**). It is specific for Pheide *in vitro* and it acts as a competitive inhibitor. This remarkable specificity is reflected by NCCs, which are derived from chlorophyll. Indeed, chlorophyll b reducing system has also been described in barley and cucumber, which could be important for the conversion of chl b to chl a during senescence (**Scheumann et al., 1999**).

2.6 GENETIC CONTROL OF SENESCENCE

The aim of molecular studies during leaf senescence is to identify genes encoding enzymes that are involved in the senescence, and also to characterize these genes to evaluate their expression levels in order to formulate an overall view of the enzymatic changes that take place during senescence. Once a senescence-associated gene has been identified, the mode of regulation of this gene can also be studied. The upstream regulatory regions of the genes can be cloned, characterized and then analyzed by comparison with other genes that show their up-regulation during senescence. The use of mutants that are defective in showing senescence, opens the way for an alternative approach for the analysis of senescence. The mutants defective in the regulation of senescence can be identified, and the signaling pathways can be dissected by comparing gene expression patterns in these mutants to identify common signalling functions (**Buchanan-Wallaston, 1997, 2003**) (Fig. 2.11).

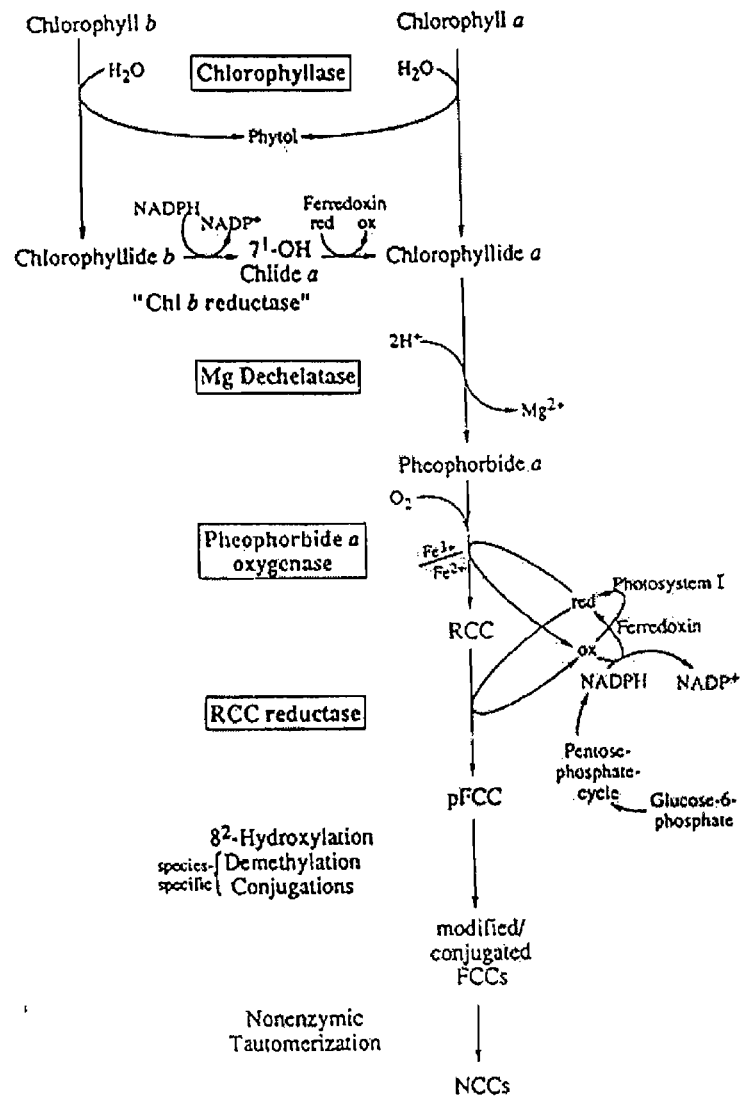


Fig. 2.10: The pheophorbide *a* (PaO) pathway of chlorophyll degradation in senescing leaves (Matile *et al.*, 1999).

during leaf senescence from a variety of plant species, such as *Arabidopsis* (Lohman *et al.*, 1994;), *Asparagus* (King *et al.*, 1995), Barley (Becker and Apel, 1993), *Brassica napus* (Buchanan-Wollaston, 1994), Maize (Smart *et al.*, (1995), Radish (Azumi and Watanabe, 1991), and Tomato (Davies and Grierson, 1989; Drake *et al.*, 1996).

It is not surprising that among SAGs , a few of them are genes encoding degradative enzymes such as RNase (Drake *et al.*, 1996), and lipases (Ryu and Wang, 1995) along with the genes involved in nutrient translocation processes such as Glutamine synthetase . Glutamine synthetase incorporates ammonium into Glycine for nitrogen recycling from senescing cells. The senescence process can also be blocked by using inhibitors of RNA and protein synthesis and by enucleation. Current molecular studies on leaf senescence are mainly focused on up-regulation of genes during senescence.

2.7 CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence has been applied as an effective and non-damaging tool for elucidating various aspects of the physiological state of the photosynthetic apparatus in intact leaves of higher plants. It can also be used to estimate the rate of photosynthetic electron transport and photosynthetic quantum yield (Sayed , 2004) as well as the capacity of thermal energy dissipation under high light stress (Demmig-Adams *et al.*, 1995).

The loss of light energy from the reaction centre as fluorescence comes primarily from the PSII reaction centre by using dark adapted chloroplast or leaves. Because, the pools of oxidation or reduction intermediates for the electron transport pathway return to a common level. Upon illumination of a dark-adapted leaf, there is a rapid rise in light emission from PSII through fluorescence followed by a series of slow oscillations. This is referred as the "Kautsky effect" (Kautsky *et al.*, 1960) (Fig. 2.12).

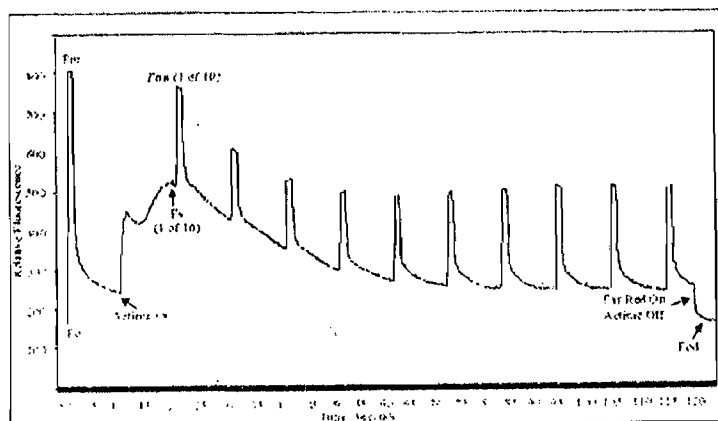


Fig. 2.12: Kautsky curve showing typical complex kinetics
([www.google.com/chlorophyll fluorescence](http://www.google.com/chlorophyll%20fluorescence))

Photosynthesis depends on the function of the light harvesting and electron transport system within the chloroplast which is indicated by the photochemical efficiency, measured as the chlorophyll fluorescence variable yield (F_v/F_{max} ratio). This can be variably correlated with the photochemical yield of the PSII. Generally, F_v/F_m ratio can be detected around 0.8 for healthy and normal plants on the basis of fluorescence induction kinetics. In case, plant suffers from any disease or abiotic stress it may reflect lower levels of the F_v/F_{max} (Spano *et al.*, 2003). The F_v/F_{max} ratio was found to be decreased progressively in flag leaves of wheat experiencing forced senescence. This loss of photosynthetic competence can result from several process including the breakdown of proteins (PSII and Rubisco) and the destruction of membranes by lipid degradation. It was found that the mutants of durum wheat retained photosynthetic competence for longer time than the control plants and therefore have a functional 'stay green' phenotype (Thomas and Smart, 1993).

PSII has been recognized as the most sensitive component of the complete photosynthetic system. The effects of high temperature on thylakoid, specifically PSII, is being through sharp rise of the basal fluorescence (F_0). The increase in F_0 occurs in plants over a small range of temperatures (40-45°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 LHCII from the reaction centre of PSII (Lootens *et al.*, 2004).

2.8 PHOTOPROTECTION: THE ROLE OF XANTHOPHYLL CYCLE

The role of xanthophylls in photoprotective heat dissipation was demonstrated by Demmig *et al.*, (1996). The xanthophyll cycle (Yamamoto, 2001) involves the conversion of violaxanthin (V) to zeaxanthin (z) via antheraxanthin (A). Thermal dissipation is correlated with the disappearance of V and the appearance of A and /or Z, and the cycle is reversed when excess light is removed (Fig.2.13).

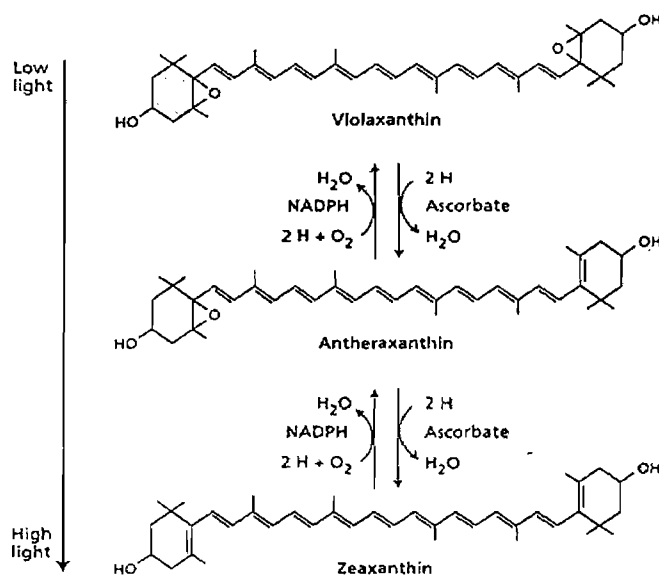


Fig. 2.13: Light dependent interconversion of Zeaxanthin (the highly quenched state of photosystem II) from violaxanthin (Taiz and Zeiger, 2003)

2.8.1 The D1 story: Damage and Recovery

Plants rely on light as essential energy source and signal for photosynthesis, biosynthesis of cell components, photomorphogenesis and development. Light requirements are largely variable in terms of intensities and spectral distribution, depending on the plant species, developmental stage, and are also affected by acclimation to local environmental conditions. For photosynthesis, photosynthetically

active radiation (PAR) from sunlight is essential. This shows large seasonal and diurnal changes, and its intensity is also influenced by meteorological factors. The higher PAR intensity favors loss for the photosynthetic capacity of chloroplast for CO₂-fixation, associated with environmental stresses. Because, the excess photons promote the production of ROS which causes the photoinhibition (PI) of photosynthesis (**Barber and Anderson, 1992; Singh and Chaturvedi, 1997; Singh, 2000; Singh, 2004; Hideg et al., 2002**).

Early research mechanisms of photoinhibition noted an associated loss of the D₁ 32 kDa-PSII reaction polypeptide (R), blockage of recovery by the protein synthesis inhibitor chloromphenicol (CAP), and protection by herbicides that get associated with Q_B binding site of the D₁ polypeptide. There is now consensus that photoinhibition involves damage and repair of protein encoded by psb A gene. Among the chloroplast genome-encoded polypeptides, D₁ shows the most rapid turnover even in low light (**Singh, 2000; Singh, 2004**) with an estimated $t_{1/2}$ of approximately 2h.

Differential regulation of protein stability seems to be a potential mechanism for modulating gene expression of D₁ turnover. It has been shown that the degradation of the D₁ protein *in vitro* is started by oxygen radicals, but the involvement of proteases has also been suggested (**Aro et al., 1994; Barber and Andersson, 1992**). Reactive oxygen species are formed during certain redox reactions and during incomplete reduction of oxygen or oxidation of water by the mitochondrial or chloroplast electron transport chains. In plant cells, chloroplasts are one of the most important intracellular generators of activated oxygen species (AOS) such as singlet oxygen (¹O₂), superoxide radicals, hydrogen peroxide and hydroxyl radicals (Fig. 2.13). AOS, especially ¹O₂ and hydroxyl radicals are highly reactive and, in the absence of protective mechanisms can cause structural and functional damage of the cell (**Asada, 2000**). These reactive molecules, especially [•]OH, are highly destructive to lipids, nucleic

acids, and proteins, which eventually leads towards impairment of the functional integrity of the photosynthetic apparatus on thylakoid membranes associated with photosynthetic electron transport chain and also inability to assimilate CO_2 to the level of biomolecules.

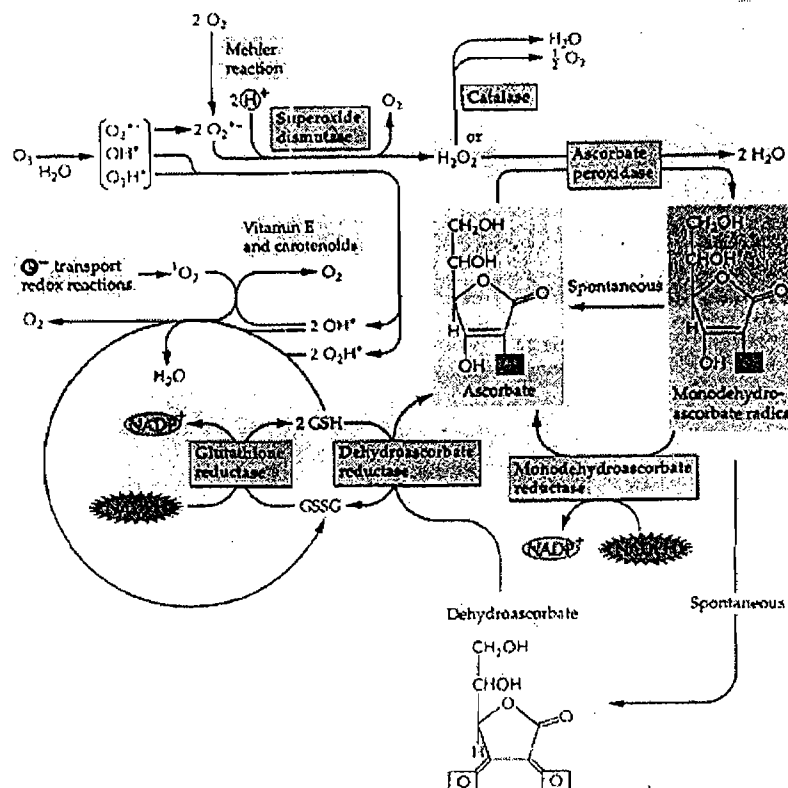
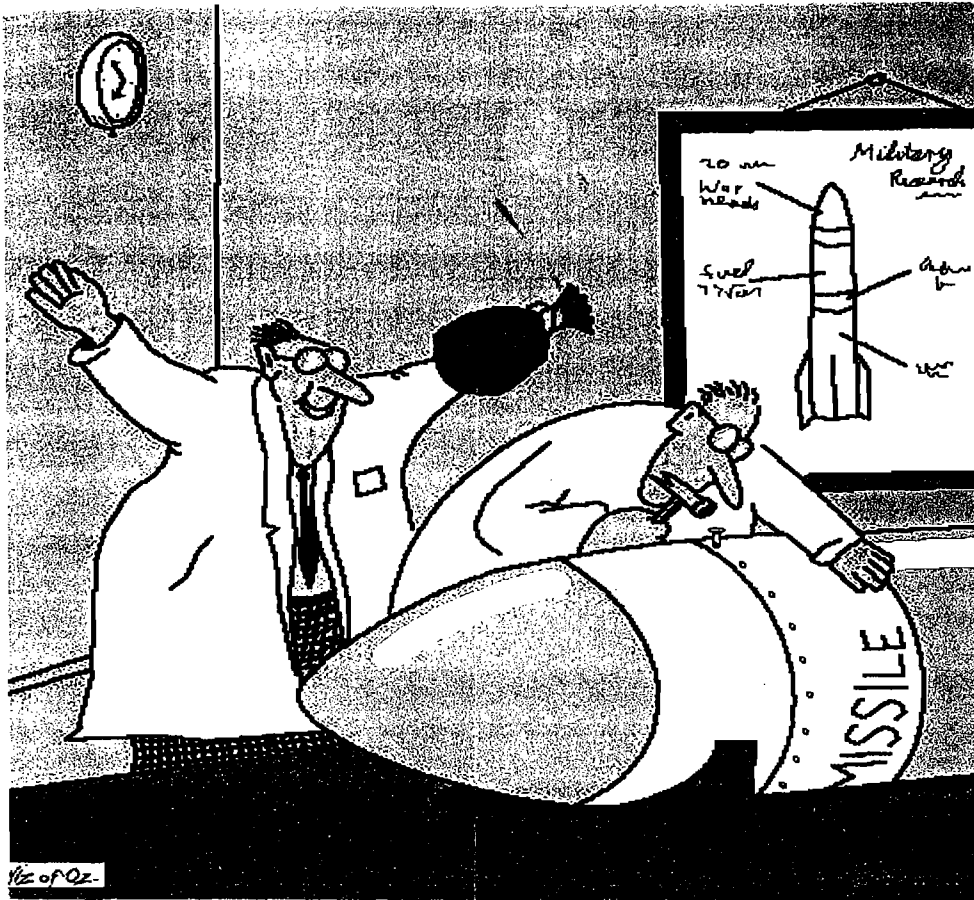


Fig. 2.13: Antioxidant defense pathway associated with various enzymes (Buchanan *et al.*, 2004)

2.9 YIELD AND YIELD ATTRIBUTES

The yield potential of cereals per unit land area is influenced by environmental variables, including light intensity and climatic temperature (Richards, 2000). Different yield parameters viz., plant height, leaf area, internodal distance, 1000-grain weight and harvest index are directly correlated in terms of productivity. Partitioning of dry matter to the grain relative to the straw is the determinant factor, as it is concerned with harvest index in the ratio of the grain to the total above-ground biomass (Nelson, 1988), despite little or no increase in total biomass (Evans, 1998).

In most wheat growing regions and especially in the Mediterranean climate grain filling is subjected to several physical and biotic stresses. The grain filling occurs under elevated temperature coupled with dry air . Consequently, kernel shriveling occurs which eventually reduces seed yield (**Singh and Malhotra, 2003**). The negative effect of rising temperature due to delayed sowing is likely to occur more in those crops which are adapted to temperate climate such as wheat and barley, as their thermal requirement for optimum growth and productivity is less compared to other crops (**Watson, 1995**). Increased temperature hastens the phenological development of the crop and reduces the grain filling period. Thus, it lowers grain yield (**Monteith, 1981; Wang et al., 1992**). The extent and magnitude of growth and yield loss in different crops under high temperature depends largely on their adaptability to various climates and the nature of carboxylation in (C₃ and C₄) as suggested by **Le Houerou et al., 1993**. Though different plant types within a crop species may also show marked variation in their optimum temperature range (**Watson, 1995; Gibson and Paulsen 1999; Yang et al 2004**).



Materials and methods.....

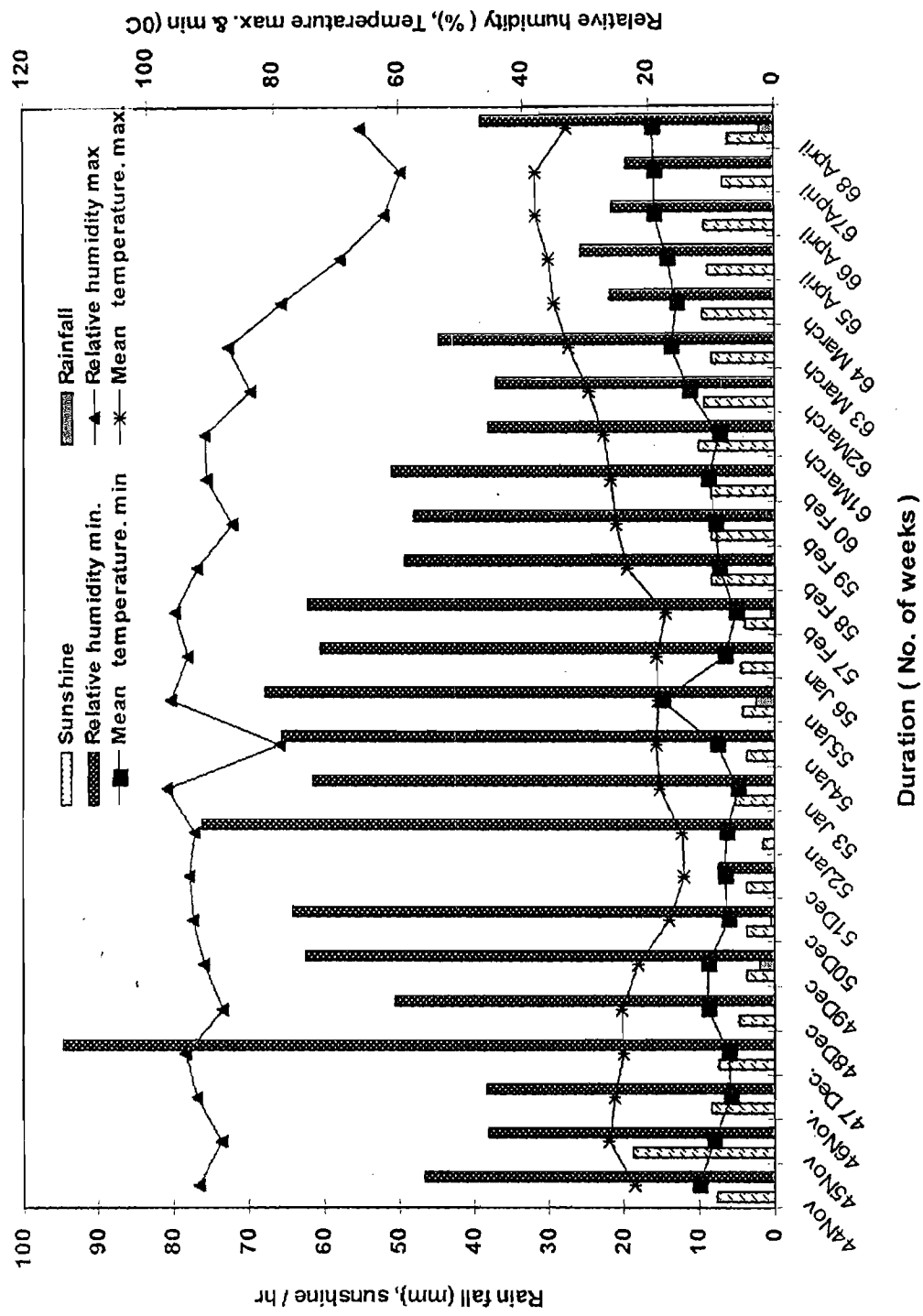


Fig.3.1: Environmental variables during the crop season November 2003- April, 2004

The experimental details regarding materials, procedures and techniques used during the course of investigation are described in this chapter.

3.1 EXPERIMENTAL SITE

All investigations were carried out during rabi season of 2003-2004 at Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Udham Singh Nagar, Uttaranchal). Geographically, the site lies in Tarai plains about 30 km southwards of foothills of Shiwalik ranges of Himalayas at 29°N latitude, 79.3°E longitude and at an altitude of 243.8 m above from the mean sea level.

3.1.1 Climate and Weather Conditions

The Tarai region experiences sub-tropical climate with hot dry summers during March-April, which continues till June afterwards followed by cool winter season from November to March almost every year. Generally, monsoon sets in the third week of June and continuous upto the end of September. The couple of shower may also be observed during winter months. The weather parameters of the year (2003- 2004) viz., like minimum and maximum temperature, relative humidity, sunshine and rainfall during the period of experimentation were recorded as shown in Fig 3.1 at meteorological observatory located at Crop Research Centre (Fig. 3.1; Appendix V).

3.1.2 Experimental Details

The field experiment was conducted during the winter season of 2003-2004 comprising two date of sowing (D1, D2) and eight varieties. All genotypes were grown under split- plot design with sowing dates in main plot and genotypes in the sub-plots of the main plot (Fig. 3.2A, B). The soil was sandy loam.

Layout of Wheat Physiology Experiment



R1															
D1				D2				D1				D2			
V ₁ 4	V ₁₀ 5	V ₁₂ 12	V ₅ 13	V ₆ 20	V ₁₅ 21	V ₁ 28	V ₈ 29	V ₅ 36	V ₁₄ 37	V ₁₆ 44	V ₄ 45	V ₇ 52	V ₅ 53	V ₃ 60	V ₁₁ 61
V ₁₁ 3	V ₂ 6	V ₆ 11	V ₁₃ 14	V ₃ 19	V ₇ 22	V ₁₁ 27	V ₄ 30	V ₁₅ 35	V ₆ 38	V ₃ 43	V ₉ 46	V ₄ 51	V ₂ 54	V ₈ 59	V ₁₆ 62
SUB				IRRIGATION				D1				D2			
V ₁₄ 2	V ₇ 7	V ₃ 10	V ₁₅ 15	V ₁₃ 18	V ₁₆ 23	V ₁₄ 26	V ₉ 31	V ₁₂ 34	V ₂ 39	V ₇ 42	V ₁₀ 47	V ₁₃ 50	V ₆ 55	V ₁₂ 58	V ₁₅ 63
V ₈ 1	V ₉ 8	V ₁₆ 9	V ₄ 16	V ₁₀ 17	V ₁₂ 24	V ₂ 25	V ₅ 32	V ₁ 33	V ₁₁ 40	V ₁₃ 41	V ₈ 48	V ₁₄ 49	V ₁ 56	V ₉ 57	V ₁₀ 64
R2															
D1				D2				D1				D2			
V ₅ 68	V ₁₀ 69	V ₁ 76	V ₂ 77	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78
R3				CHANNEL				D1				D2			
V ₁₀ 68	V ₁ 69	V ₄ 76	V ₂ 77	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78	V ₅ 67	V ₁₂ 70	V ₃ 75	V ₈ 78
V ₉ 66	V ₆ 71	V ₁₅ 74	V ₁₁ 79	V ₉ 66	V ₆ 71	V ₁₅ 74	V ₁₁ 79	V ₉ 66	V ₆ 71	V ₁₅ 74	V ₁₁ 79	V ₉ 66	V ₆ 71	V ₁₅ 74	V ₁₁ 79
V ₇ 65	V ₁₄ 72	V ₁₆ 73	V ₁₃ 80	V ₇ 65	V ₁₄ 72	V ₁₆ 73	V ₁₃ 80	V ₇ 65	V ₁₄ 72	V ₁₆ 73	V ₁₃ 80	V ₇ 65	V ₁₄ 72	V ₁₆ 73	V ₁₃ 80
V ₁₀ 82	V ₃ 81	V ₈ 88	V ₁₂ 89	V ₁₀ 82	V ₃ 81	V ₈ 88	V ₁₂ 89	V ₁₀ 82	V ₃ 81	V ₈ 88	V ₁₂ 89	V ₁₀ 82	V ₃ 81	V ₈ 88	V ₁₂ 89
V ₇ 84	V ₁₆ 85	V ₁₃ 92	V ₅ 93	V ₇ 84	V ₁₆ 85	V ₁₃ 92	V ₅ 93	V ₇ 84	V ₁₆ 85	V ₁₃ 92	V ₅ 93	V ₇ 84	V ₁₆ 85	V ₁₃ 92	V ₅ 93

R = 3 Replication (R₁, R₂ & R₃)

D = 2 Date of sowing D₁ 25-11-03

D₂ 19-12-03

V = 16 Variety

V₁ VL 802 V₂ VL 616
V₃ VL 738 V₄ VL 804
V₅ UP 2338 V₆ UP 2425
V₇ UP 1109 V₈ UP 2382
V₉ UP 2113 V₁₀ PBW 396
V₁₁ PBW 343 V₁₂ PBW 373
V₁₃ PBW 175 V₁₄ HD 2687
V₁₅ C 306 V₁₆ RAJ. 3765

Plot size 5.0X2.5 m² (Seed 100 kg/ha, row spacing 23 cm.)

Wheat Physiology Treatment:



Factor A	Date of sowing (two)	
	1. 27-11-03	D ₁
	2. 20-11-03	D ₂
Factor B	Varieties (5)	
	1. VL 804	V ₁
	2. VL 829	V ₂
	3. VL 616	V ₃
	4. VL 421	V ₄
	5. VL 738	V ₅

D ₁		R ₁	D ₂		D ₁		R ₂	D ₂		D ₁		R ₃	D ₂	
V ₁			V ₃		V ₅			V ₂		V ₂			V ₄	
5			6		15			16		25			26	
V ₄			V ₅		V ₁			V ₄		V ₄			V ₁	
4			7		14			17		24			27	
V ₅			V		V ₅			V ₁		V ₅			V ₁	
3			8		13			18		23			28	
V ₅			V ₄		V ₂			V ₃		V ₁			V ₂	
2			9		12			19		22			29	
V ₁			V ₂		V ₄			V ₅		V ₃			V ₅	
1			10		11			20		21			30	

Road

Replication	3(R ₁ , R ₂ & R ₃)
Date of sowing	D ₁ = 27-11-03 D ₂ = 20-12-03
Seed rate	100 kg/ha
Plot size	4X2.5 m ² (10 rows each plot)

Year 2003-2004**(a) Date of sowing Main plot**1. 25.11.2003 : D₁2. 19.12.2003 : D₂**(b) Varieties Sub plot**1. VL-616 : V₂2. UP-2338 : V₅3. UP-2425 : V₆4. UP-2113 : V₉5. PBW-343 : V₁₁6. PBW-175 : V₁₃7. HD-2687 : V₁₄8. VL-421 : V₄

The characters of the varieties are given below:

1. Temperature sensitive

UP-2425

UP-2338

PBW-343

HD-2687

2. Rainfall

PBW-175

UP-2113

3. Hilly varieties

VL-421

VL-616

3.1.3 Experimental Design and Layout

Experiments were conducted under split plot design with three replications. Date of sowing was maintained with main plot and various genotypes in the subplots of a main plot.

Chemicals and Glasswares

Chemicals were obtained from Sigma, Hi-media, BDH, Qualigens, Merck and SRL. Anti-rabbit goat immunoglobulin IgG conjugated with alkaline phosphatase were purchased from Genie,

Bangalore. All the glasswares used for the present study were obtained from Borosil.

3.1.4 Cultural Operations

Seedbed preparation

Pre-sowing irrigation was given 10 days prior sowing in order to create ideal conditions for good germination. The field was prepared by tractor drawn plough followed by disc harrow and levels.

Sowing

All wheat genotypes were sown using seed at the rate of 100 kg/ha of the varieties used in the experiment as per the date of planting in rows. Seeds of the above varieties were treated with fungicide vitavax@2.0 g/kg seeds).

Fertilizer application

The crop was fertilized with 120 kg N/ha, 60 Kg P_2O_5 /ha and 40 Kg K_2O /ha. Half of the nitrogen and full dose of P_2O_5 and K_2O were applied as basal before sowing and the remaining half of the nitrogen was split into two (one fourth at first irrigation and remaining one fourth at ear head emerging stage). Nitrogen was applied through urea (46% N), phosphorous through single super phosphate (16% P_2O_5) and potassium through M.O.P. (60% K_2O).

3.1.5 Irrigation and Weed Control

Irrigation was provided whenever necessary particularly during all the critical stage of crop growth to maintain adequate soil moisture. To control weeds, isoproturon was sprayed @ 0.75 kg/ha in 800 – 1000 litre of water per hectare during 32-35 days after sowing. The weeds which could not be controlled by chemical method, were also removed with the help of khurpi as and when necessary to maintain weed free situation.

3.1.6 Harvesting and Threshing

At maturity, one row from both the side in length and 0.5 m from both sides in width were taken as border area. The crop was harvested (after removing the crop from border area), tied into bundles and tagged. These bundles were allowed to dry in the sunlight for 4-5

days. Threshing of bundles from the individual plots was done using power thresher, and grain yield was recorded.

3.2 MORPHOLOGICAL CHARACTERISTICS

3.2.1 Plant Height and Internodal Distance

The shoots height was recorded from the ground level to the top of the plant after emergence of ear along with internodal distance. The average of the different parameters were expressed in centimeters.

3.2.2 Leaf Area

The leaf was calculated using leaf area meter (portable area meter). Leaves were taken from 1st, 2nd, 3rd, 4th, 5th, and 6th position after attaining the full expansion (after anthesis) from the mother shoots along with their replicates. Leaf area was expressed in cm²/plant.

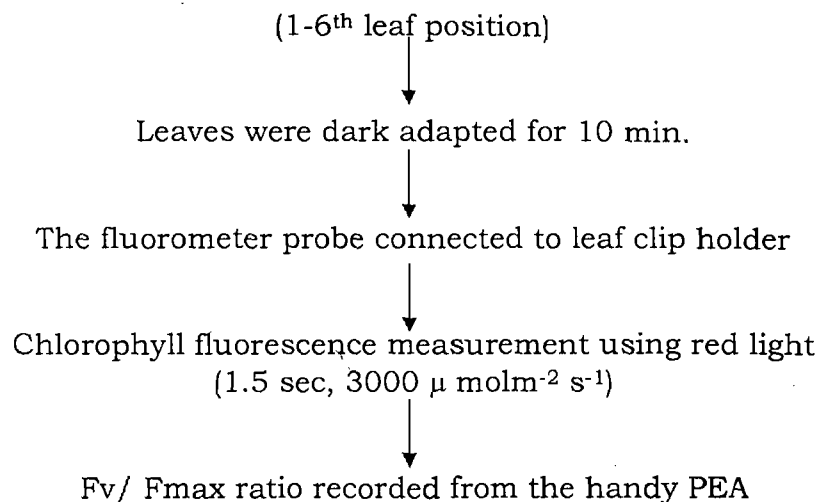
3.2.3 Spike Length

Length of ten spikes per treatment was measured. The average spike length was reported as centimeter per spike.

3.3 CHLOROPHYLL 'a' FLUORESCENCE VARIABLE YIELD (Fv/Fmax)

Chlorophyll 'a' fluorescence emitted by green plants reflects photosynthetic ability of PS-II. A handy plant efficiency analyzer (Handy, PEA, Hansatech, UK) was used to monitor (Fv/Fmax). Measurements were taken at 0, 10, 20 and 30 days after anthesis using various leaves (1-6). This includes flag leaf (6th) and ear head. All the measurements were recorded in the forenoon hrs. (9-10 am) in between to avoid photo inhibition. The minimal fluorescence level (F₀) with open PSII reaction centres was determined by measuring under modulated light, not to induce any significant variable fluorescence. The maximum fluorescence level (F_m) of closed PSII centre was determined by providing 1.5 sec. saturating pulse at 300 $\mu\text{mol m}^{-2}\text{s}^{-1}$ on dark adopted leaves by using following protocol:

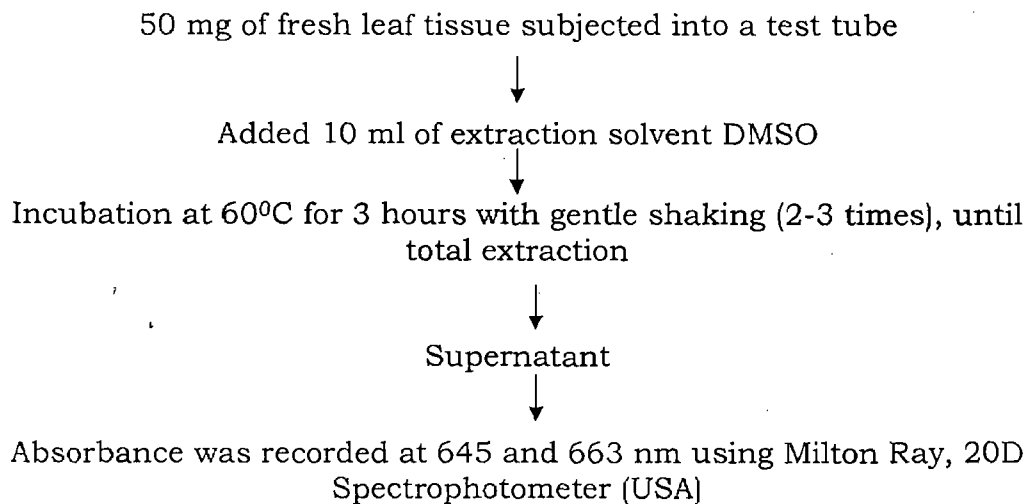
Leaf clips were attached to the mid portion of each leaf, ear/ head



3.4 BIOCHEMICAL PARAMETER

3.4.1 Chlorophyll Estimation

The chlorophyll content was estimated by harvesting flag leaf at 0, 10, 20, 30 days after anthesis by using dimethyl sulphoxide (DMSO) extraction procedure as described by **Hiscox and Israelsham (1979)** as per following protocol.



Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of leaf tissue were calculated according to (**Hiscox, 1979**) by using the following equation:

(a) Chlorophyll 'a' (mg/g fresh weight)

$$= (12.7 \times A_{663} - 2.63 \times A_{645}) \frac{V}{1000 \times w}$$

(b) Chlorophyll 'b' (mg/g fresh weight)

$$= \frac{(22.9 \times A_{645} - 4.48 \times A_{663}) \times V}{1000 \times w}$$

(c) Total chlorophyll (mg/g fresh weight)

$$= \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{1000 \times w}$$

3.5 ENZYMATIC ACTIVITY

3.5.1 Peroxidase Activity (Usepa, 1994)

Reagents

The following reagents were prepared to monitor peroxidase activity

1. 200 mM potassium phosphate buffer (pH 7.0)
2. 0.17 mM hydrogen peroxide-prepared by diluting 1 ml of 30% hydrogen peroxide to 100 ml with reagent grade water. Further diluted 1 ml of this solution to 50 ml with 200 mM potassium phosphate buffer (freshly prepared).
3. 0.25 mM 4-amino antipyrine with 170 mM phenol, prepared by dissolving 810 mg phenol in 40 ml reagent grade water. Further 25 mg 4-aminoantipyrine and dilute to a final volume of 50 ml with reagent grade water.

Procedure

The enzyme reaction mixture was prepared in the cuvette itself to monitor enzyme activity based on oxidation and reduction. The change in optical density of the reaction mixture was measured spectrophotometrically at 510 nm. The reaction mixture was prepared as stated below:

i. Phenol/ amino antipyrine solution	1.4 ml
ii. H ₂ O ₂ (0.0017M)	1.5 ml
iii. Enzyme extract	0.1 ml
Total	<u>3.0 ml</u>

The reaction mixture was kept at 25°C for 3-4 min, subsequently enzyme was added and mixed well prior recording the enzyme activity spectrophotometrically at OD 510 nm.

Calculate $\Delta A_{510} / \text{min}$ from linear portion of the curve.

Calculation

$$\text{Units/ mg} = \frac{\Delta A_{510} / \text{min}}{6.58 \times \text{mg enz/ ml reaction mixture}}$$

3.5.2 Superoxide dismutase activity

Beauhamp and Fridovich (1971) with some modifications

SOD activity was assayed by its ability to inhibit the reduction of nitro blue the tetrazolium (NBT) by the $\text{O}_2^{\cdot -}$ generation system xanthine oxidase producing an increase in absorbance at 560 nm of about 0.02 units/ min at 25°C in the absence of enzyme that cause 50% inhibition of the initial rate of NBT reduction.

Procedure

Seedlings (0.2 gm) were grinded in 5 ml chilled phosphate buffer (0.1 M, pH = 7.8)/ 4°C

↓
Centrifuged 10,000 g/ 20 min/4°C

↓
Supernatant was chosen as enzyme extract

Reaction mix

Phosphate buffer (pH 7.8)	50 mM	0.8 ml
Methionine	0.1 mM	0.3 ml
EDTA	0.5 M	0.3 ml
NBT	75 μM	0.3 ml
Riboflavin	2 μM	0.3 ml
Enzyme extract		25 μl

In 2 ml of reaction mix, 25 μl enzyme extract was added. Reaction was started by placing tubes below two 15 W fluorescent lamps/10 min. Reaction was stopped by keeping the tubes in dark/ 10 min. Absorbance was taken at 560 nm.

3.6 MELANODEALDEHYDE (MDA) ASSAY

The extent of lipid peroxidation in leaves was estimated by measuring the amount of MDA by the method described by **Heath and Packer (1968)** which takes into account the possible influence of interfering compounds in the assay for TBA-reactive substance.

Procedure

Wheat flag leaf (0.1 gm) + 1 ml of TBA (0.25%) in 10% TCA

↓
Grinded

↓
Homogenate / 95°C / 30 min.

↓
Centrifuged 10,000 rpm / 30 min

↓
Supernatant, absorbance at 532 and 600 nm.

↓
The MDA content was calculated using following equation

$$= \sum (532 \text{ nm} - 600 \text{ nm}) = 1.55 \times 10^5 \text{ (M}^{-1} \text{) (CM}^{-1} \text{)}$$

3.8 FLAG LEAF SENESCENCE ALONG WITH PLANT AND EAR-HEAD SENESCENCE/MATURITY

To monitor senescence parameters five shoots from each replicate were chosen to observe the on-set of the senescence behaviour at 0, 10, 20 and 30 days after anthesis. The different formulas used to monitor senescence are given below:

$$(a) \% \text{ senescence Flag leaf} = \frac{\text{TGA} - \text{TSA}}{\text{TGA}} \times 100$$

Where, TGA = Total green area of the flag leaf

TSA = Total senescence area of the flag leaf

$$(b) \% \text{ senescence (whole plant)} = \frac{\text{TL} - \text{GL}}{\text{TL}} \times 100$$

Where TL = total leaf area

GL = total green leaf area

$$(c) \% \text{ ear head senescence (maturity)} = \frac{\text{TBA} - \text{TGA}}{\text{TBA}} \times 100$$

(Observed visually)

Where TBA = Total brown appearance in ear head

TGA = Total green appearance in ear head

3.8 ULTRASTRUCTURE OF CHLOROPLAST AT DIFFERENT STAGES OF SCENESCENCE THROUGH TRANSMISSION ELECTRON MICROSCOPY

TEM was done in All India Institute of Medical Sciences (AIIMS), New- Delhi

3.8.1 Reagents and solutions used for TEM

Observations

- (i) **Fixative:** Glutaraldehyde (1%) with p-formaldehyde (2%) solution freshly made in 0.1 M phosphate buffer (pH 7.4, 0.1 M).
- (ii) **Karnovsky's fluid:** Glutaraldehyde (1%) with para-formaldehyde (4%) made in 0.1 M sodium phosphate buffer (pH 7.4).
- (iii) **Osmium tetroxide (2%):** Made in double distilled water, stored at 4°C in brown colored bottle.
- (iv) **Dry acetone:** Prepared by adding sufficient amount of anhydrous copper sulphate and filtered before use.
- (v) **Embedding medium:** Prepared by mixing Araldite (CY 212), and Dodecyl succinic anhydride (each 10 ml), trimethylamine-methyl phenol (0.4 ml) and plastilizer (1 ml).
- (vi) **Uranylacetate:** Saturated solution of uranyl acetate prepared in ethanol (50%) was filtered before use and used fresh always.
- (vii) **Lead citrate:** Prepared by using half a pellet of NaOH dissolved in double distilled water (12 ml). The lead citrate (50 mg) was added and shaken well, centrifuged (2 min) before use. The solution was used fresh.

(viii) **Toluidene blue:** Solution (1%) was prepared by using toluidene blue (1 g) in borax (1%) solution using distilled water and final volume adjusted to 100 ml.

(ix) **Formvar or colloidal solution:** Collodian or formvar (1-3%) was prepared in chloroform or ethylene dichloride.

The different steps to process leaf sections for ultrastructure studies are stated below:

i) Pre fixation: The tissue was placed in the fixative (2-24 hr) at 4°C. This step was performed to prevent the autolysis, change in volume and shape of various chemical constituents of the cell.

ii) Washing: After fixation the specimens were washed thoroughly (4°C) with phosphate buffer saline (PBS) to remove excess fixative. Because traces of fixative, if left in the specimen, may react with the secondary fixative.

iii) Post fixation (Secondary fixation): Osmium tetroxide (2%) solution was used as the post fixative. The leaf tissues were kept in post fixative for 6 hr.

iv) Washing: After secondary fixation leaf tissue was rinsed thoroughly with phosphate buffer (0.1 M, pH, 7.4, 6 min, 4°C) to remove excess fixative.

v) Dehydration: The plant material was dehydrated with ethanol for different time intervals as per the following schedule.

Sl. No.	Absolute alcohol (ml)	Water (ml)	Time (min)
1.	30	70	30
2.	50	50	30
3.	70	30	30
4.	90	10	30
5.	100	0	30
6.	100	0	30

vi) Clearing: After dehydration the tissue was immersed in propylene oxide (60 min, room temperature).

vii) Infiltration: The plant material was infiltrated by gradually decreasing the concentration of clearing agent and proportionately increasing the concentration of embedding medium as per the following schedule:

S. No.	Propylene oxide (ml)	Embedding medium (ml)	Time (min.)
1.	75	25	36
2.	50	50	36
3.	25	75	36
4.	0	100	24-36

viii) Embedding: It was done by using embedding medium and flat embedding mould.

ix) Polymerization: The sample was polymerized by gradually increasing the temperature from 37°C to 60°C with an increase of 7-8°C every 24 hr. After polymerization the blocks were removed by bending the mould side ways.

Ultramicrotomy

(i) Thinning of blocks: After fixing the block onto the holder it was thinned with a clean razor blade to produced a pyramidal form at the tip of the block.

(ii) Preparation of semithin section for optical microscopy: Before ultrathin sectioning, thick sections (0.5-2.0 μm) were cut for scanning the tissue under an optical microscope. The semithin sectioning was done to specify the state of the embedded specimen, the fixation and mainly for the selection of the area for ultrathin sectioning, the size and position of the cutting face for final trimming. The slide with sections was placed on a hot plate at 80°C and dried. The sections were examined with toluidene blue for 30-45 sec. The slides were washed thoroughly with distilled water and after drying were mounted with cover slips. The slides were examined with toluidene blue for 30-45 sec. The slides were

washed thoroughly with distilled water and after drying were mounted with cover slips. The slides were observed under microscope.

Preparation of ultrathin sections

After scanning the sections under optical microscope the area to be examined under TEM was selected and blocks were further trimmed. The ultrathin sections floating on water were stretched by exposing to chloroform vapours. Grids made of copper and coated with 3 per cent formvar (poly vinyl formaldehyde) in ethylene dichloride was used for lifting the sections.

Staining ultrathin sections

Uranyl acetate and lead citrate was used to obtain a good contrast. The grid carrying the sections was placed onto stain for 10-15 min and then washed twice using ethanol (50%) and double distilled water with continuous agitation and dried with filter paper. Afterwards the grid was placed in lead citrate for 5-10 min and washed within sodium hydroxide (0.02 M) and washed twice by using distilled water. The grid was dried and stored in grid box. The sections were observed in transmission electron microscope. Complete protocol for TEM is given in Table 3.1.

3.9 PROTEIN ANALYSIS BY SDS-PAGE

To find out the changes in total protein profiles associated with the different stages of senescence, protein analysis was done by one dimensional sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli buffer system (Laemmli, 1970).

Principle

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used method for analyzing protein mixes qualitatively. SDS is an anionic detergent which provides the same negative charge to all the protein. The method is based on the separation of protein according to size under the force of electric field.

$$\text{Electrophoretic mobility } \mu = \frac{V}{E} = \frac{q}{f}$$

Table 3.1: Protocol for TEM

Sl. No.	Description	Time	Temperature (°C)
1.	Prefixation (Glutaraldehy (1%) + Paraformaldehyde (2%))	Overnight	4
2.	Washing Phosphate buffer (0.1 M, pH 7.4)	15 min. × 3	4
3.	Post fixation osmium tetroxide (2%)	6 min	4
4.	Washing phosphate buffer (0.1 M, pH 7.4)	30 min × 3	4
5.	Dehydration		
	Etahnol (30%)	30 min	4°C
	Etahnol (50%)	30 min	4°C
	Etahnol (70%)	30 min	4°C
	Etahnol (90%)	30 min	4°C
	Etahnol (100%)	30 min	4°C
	Etahnol (100%)	30 min	Room temperature
6.	Clearing		
	Propylene oxide	60 min	Room temperature
7.	Infiltration		
	Propylene oxide : Embedding 3 : 1 (75 + 25 ml)	36 min	Room temperature
	2 : 2 (each 50 ml)	36 min	Room temperature
	1 : 3 (25 + 75 ml)	36 min	Room temperature
8.	Embedding-Embedded in pure embedding mixture		
9.	Polymerization	Time	Temperature
		24 hr	37°C
		24 hr	50°C
		36 hr	55°C
		50 hr	60°C

V = velocity of the molecule

E = electric field

q = Change on the molecule

f = the frictional coefficient

Qualitative estimation of protein

I. Reagents (stock solution)

1. Acrylamide-Bisacrylamide solution 30%

29.2 g acrylamide and 0.8 gm of bisacrylamide were dissolved in 100 ml of dH₂O. The solution was filtered through 0.45 μ M filter and stored in dark bottle /4°C for not more than 30 days.

2. Running gel buffer (1/5 M Tris, pH 8.8) 4× conc.

45.5 gm Tris was dissolved in 150 ml of ddH₂O and pH 8.8 with 1 N HCl. Volume was made upto 250 ml with ddH₂O, filtered through 0.45 μ M filter and stored / 4°C.

3. Stacking gel buffer (0.5 M Tris pH 6.8, 4x conc.)

15.25 gm Tris was dissolved in 150 ml of ddH₂O and pH 6.8 with 1 N HCl. Volume 250 ml filtered 0.45 μ m filter and stored at 4°C.

4. SDS, 10% solution – 5 gm SDS was dissolved/ 50 ml dH₂O, stored at R/T.

5. SDS sample buffer 6 x conc.

To 7,ml of 4 x conc. Tris-Cl/SDS buffer (0.5 M, pH 6.8), 3 ml of glycerol, 1 gm SDS, 0.6 ml of mercaptoethanol and 1.2 mg of bromophenol blue was added and stored 0.5ml aliquots at - 70°C.

6. SDS/electrophoresis buffer (reservoir buffer, 5x conc.)

15.1 gm of Tris base, 72 gm of glycine and 5 gm of SDS were dissolved in dH₂O and volume made upto 1 lit and stored at 4°C. For use as working solution it was diluted to 1x conc. by mixing 1 part of buffer with 4 parts of water.

7. APS (10%)

100 mg of APS was dissolved in 100 ml of dH₂O, prepared fresh each time.

8. **Sample extraction buffer (0.6 M Tris, pH 6.8)**

7.26 gm Tris based was dissolved 150 ml of dH₂O and pH 6.8 with 1N HCl volume 100 ml, 4°C store.

9. **Staining solution**

0.625 g of CBB R-250 was dissolved in 250 ml CH₃OH and 100 ml glacial acetic acid. The final volume 500 ml with dH₂O and filtered through Whatman No. 1 filter paper.

10. **Destaining solution**

100 ml of CH₃OH and 70 ml of glacial acetic acid were mixed with dH₂O and final volume was made upto 1 lit with water.

11. **Fixing solution**

150 ml methanol was added to 250 ml dH₂O. To this solution, 57 gm of trichloroacetic acid and 17 gm of sulphosalicylic acid were added and dissolved completely.

Preparation of samples for loading on to SDS-PAGE

50 µl sample + 50 µl crude leaf extract/ protein



Kept at boiling temperature/ 10 min.



Spin. briefly



Loaded

Sample loading and electrophoresis

The stacking gel and resolving gel were made 5% and 12.5% acrylamide respectively (Appendix). Sample (loading size/ lane, 100 µl = 12.5 mg crude soluble protein) was loaded into each well in the stacking gel. Electrophoresis was carried out at a constant current of 10 mA till the sample traveled stacking gel and then the current

increased 10 mA to 25 mA till the dye reached the bottom end of the resolving gel. The room temperature was maintained 25°C during the electrophoresis.

Transfer of protein gel from the SDS-PAGE to solid support : Western blotting

The term 'western blotting' was given by Burmett in 1981. This is procedure which does not directly involve nucleic acids but which is of importance in gene manipulation and analysis of protein.

Principle

The principle involves the transfer of electrophoresed protein bands from a polyaculamide gel on to a membrane of nitrocellular on nylon, to which they bind study. The bound proteins are available for analysis by a variety of specific protein legand interaction. Most commonly, antibodies re used to detect specific antigen.

Reagents

(I) Electron transfer buffer

Tris	25 mM
Glycine	192 mM
Methanol	20%
SDS	0.01%

Made upto 1 litre with distilled water pH 8.3

1. 1 X TTBS

Tris
Sodium-chloride

Twin-20 (20% w/v)

Made up to 1 lit with DW, pH 7.6

2. Blocking buffer

3. Primary absorbance solution

Detection

1. Transferred the gel band's on to the nitrocellulose membrane with the help of transfer assembly.
2. Blocked with BSA solution, overnight at R/T.
3. Washed with TTBS (15 min×1 and 5 min × 2)

4. Shaked in primary antibody for 1 hr. R/T.
5. Washed with TTBS (1 min $\times$ 1, 5 min $\times$ 2).
6. Treated with secondary Ab for 45-60 min R/T (secondary antibody was diluted 30,000 fold in TTBS).
7. Washed with TTBS (15 min $\times$ 1, and 5 min $\times$ 4).
8. Treated with required amount of BCIP/NBT substrate for detection of specific protein.

Silver staining for protein

- | | |
|----------------------|--|
| 1. Fixing solution | 40% methanol, 10% acetic acid |
| 2. Washing solution | 30% ethanol |
| 3. Sensitization | 0.02% $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{ H}_2\text{O}$ |
| 4. Staining solution | 500 mg/500 ml |
| 0.2% AgNO_3 | 100 μl |
| 0.02% Formaldehyde | Company stock bottle. |
| 5. Developer | 3% Na_2CO_3 |
| | 0.05% Formaldehyde |
| | 0.5% sodium thiosulphate |
| 6. Stop | |
| Trizma base (Sigma) | 25 gm/500 ml |
| Acetic acid | 12.5 ml |
| 7. Drying solution | 25% ethanol |
| | 2% Glycerol |

Procedure

2. Fixed the gel in fixing solution for 60-90 min.
3. Washed with 30% ethanol 3 $\times$ 20 min.
4. Sensitization with sodium thiosulfate reagent (1 min only).
5. Reused by TDW 3 $\times$ 20s.
6. Staining in silver nitrate solution 20 min.
7. Reused by TDW 3 $\times$ 20s.
8. Developer (3-4 min.)
9. Reused TDW 2 $\times$ 30s.
10. Stop solution 5 min.
11. TDW 2 $\times$ 30 min.

3.10 YIELD AND YIELD ATTRIBUTES

The spikes were harvested from 1.0 m row length at the time of maturity for recording attributes.

3.10.1 1000-grain weight

Random samples of the grains from individual genotypes were obtained. These samples were used to detect 1000-grain weight after drying the grains at room temperature (30°C) to minimize intrinsic moisture content uniformly (1000 grains were counted from each plot and weight was recorded in grams).

3.10.2 Biological yield

The crop from each plot was harvested, bundled, labeled and dried in the field for 4-5 days. Bundles were weighed before threshing to record the biological yield, which was presented as q/ha.

3.10.3 Grain yield / economic yield

After threshing and winnowing, grain yield per net plot was recorded Kg/ plot and finally expressed as q/ha.

3.10.4 Harvest index

The harvest index was calculated from the ratio of economic yield to total biological yield as stated below:

$$HI (\%) = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

3.10.5 Anthesis scheduling

Anthesis scheduling was observed keeping the ear emergence criterion in consideration. The anthesis was recorded with the value of almost 100% upon full emergence of ear and acquisition of pollens.

3.10.6 Statistical Analysis

The allocation of treatments to the plot was done as per split plot design. The data on the different characters were analyzed by using the analysis of variance technique for a split plot design (**Panse and Sukhatme, 1967**). The treatment comparisons were made at 5 per cent level of significance.



Results.....

Plants are exposed to different kinds of stresses throughout their life span and various metabolic processes are affected under adverse environmental variables (**Levitt, 1972; Agarwal and Pandey, 2003**). The growth and development of wheat (*Triticum aestivum*, L.) crop are governed by genetic characters largely depend upon a number of environmental factors, may also be associated with different sowing dates (**Saini et al., 1988**). Generally, sowing of wheat is delayed under rice -wheat cropping system which impairs yield oftenly, though the magnitude of yield reduction varies with the varieties. The different environmental variables specifically elevated temperature interacts with growth and reproductive phases over the long period of crop growth in determining the magnitude of reduction. Therefore, temperature plays an important role in determining sowing time and consequently the duration of different phenophases, and eventually the crop productivity (**Tiwari and Singh, 1993**).

4.1 GROWTH PARAMETERS

4.1.2 Shoot Length (cm)

Data pertaining to shoot length during the year (20 03-2004) is given in (Fig.4.1, App. IA) and their analysis of variance in (App. VI A). The data clearly revealed that taller shoots were recorded in case sown early (D1) as compare to delayed sown i.e.,(D2), as well as it was found significantly higher in timely sown cultivars as compare to late sown one. The intervarietal difference of shoot lengths was also found to be significant. The longest shoot length was found (84.88cm) in variety VL-616 and was significantly higher over rest of the varieties except UP-2338. The percentage reduction in shoot length was found to be maximum in UP-2113 (15.60%), followed by UP-2338 (9.07%) incase sowing delayed for about two weeks. The cultivars PBW-343(2.14%) was less affected in expressing their shoot length followed by VL-421(3.68%) and UP-2425(4.79%).

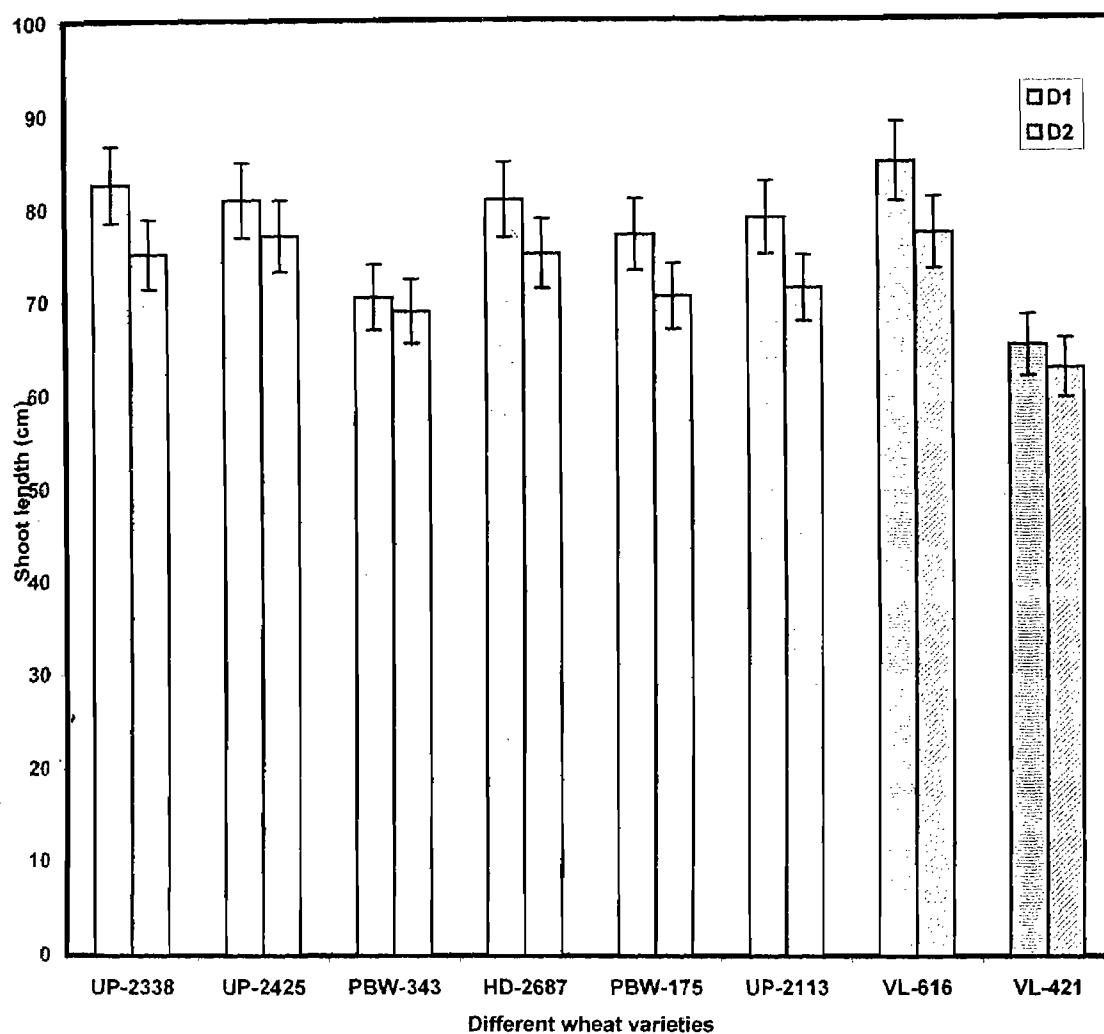


Fig. 4.1: Effect of differential dates of sowing on plant height of different wheat cultivars. D1 and D2 indicates timely and delayed sown schedules. Data represents the mean values of five independent measurements

4.1.3 Spike Length

The data shown in Fig 4.2 and App. IB indicates that the length of spike was found to be longer in case the cultivars were sown early or in time. Almost all genotypes opted similar pattern for their spike length in case sown differentially. UP-2338 has shown maximum spike length and is also significantly higher from rest of the genotypes. Minimum percentage reduction of spike length was observed in UP-2113 (7.40%) followed by VL-421 (7.8 %) and UP-2338 (8.09 %) while rest of the genotypes viz., PBW-343(12.9%), UP-2425 (10.67%), PBW-175 (10.30%), HD-2687 (10.47%) and VL-616(10.18 %) have shown higher values for the reduction in their spike length (App VI B).

4.1.4 Leaf Area

Leaves are the most important part of a plant because they phototransform light energy in the form of photosynthetase and to supply them to different sink parts of a plant. The more the area, more will be the photosynthesis i.e. biosynthesis of biomolecules. The data shown in Fig 4.3 and App IC clearly shows that timely sown cultivars express more leaf surface area as compare to late sown and it shows significant higher value in all the five leaves . The flag leaf has shown maximum leaf area (Fig 4.3 A,B,C) in all the genotypes, amongst all the five leaves. UP-2113 has significantly higher leaf area over the rest of the varieties (App. VI C). The minimum value of flag leaf area was shown by VL-421 followed by PBW-175, UP-2425 and HD-2687 (App IC). In terms of percentage reduction, there is significant variation in different leaf positions as well as different genotypes. In the case of leaf position 1 (basal leaf) , percentage reduction in leaf area was maximum in PBW-343(40.17%) followed by VL-421 (36.48%) while minimum reduction was recorded in UP-2338 (8.21%). The maximum percentage reduction in leaf area at position 2, 3 and 4 was observed in VL-421(60%), PBW-343(38.32%) and VL-421 (25.6%) respectively. The flag leaf (at position 5th) which has shown maximum leaf area amongst all other leaves , the

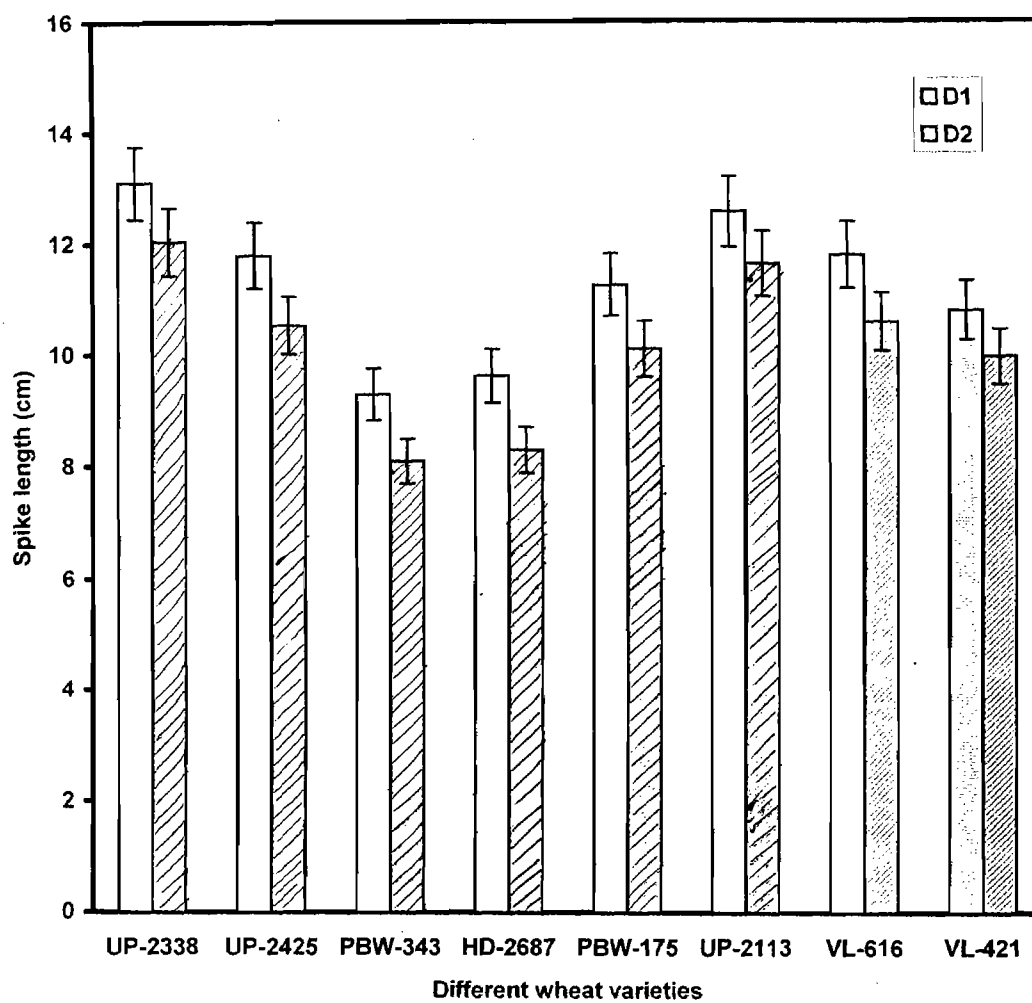


Fig. 4.2: Effect of differential dates of sowing on spike length in different wheat cultivars. D1 and D2 indicates timely and delayed sown schedules. Data are mean of five independent measurements

Photoplate 4.2: Effect of date of sowing on spike length in different wheat cultivars under D1 (timely) and D2 (delayed) sown. A. UP-2338, B. UP-2113 and C. VL-616



A



B



C

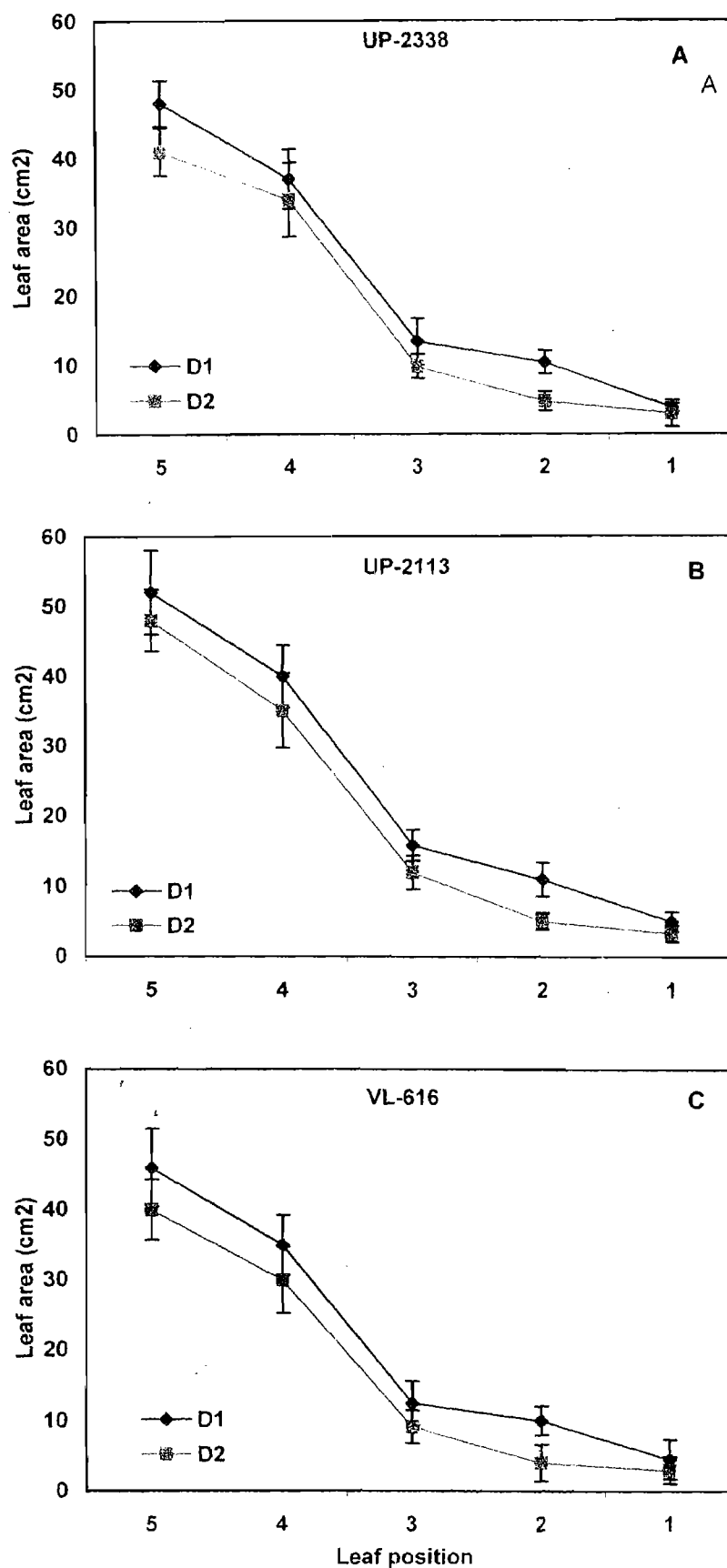


Fig. 4.3: Leaf area of plants grown under different date of sowing the flagleaf represents leaf position 5. D1 and D2 indicate about time and delayed sown schedules. The values are the mean of five independent measurements.

percentage reduction was maximum in VL-421(18.75%) followed by PBW-343 (18.42%) and PBW-175(15.15%) , while minimum reduction was shown by UP-2113 (7.6%) followed by VL-616(13%) and UP-2338 (14.5%).

4.1.5 Internodal Distance

It is the distance between two nodes, or the distance between two successive leaves. Different Internodal distance were found highly affected by date of sowing, as late sown cultivars have shorter distance compared to timely sown one (Fig. 4.4 A,B,C and App I D). All varieties have shown differential internodal distance. The first internodal distance i.e., distance between flag leaf and successive leaf was found maximum (19.5cm) in VL-616 , while it was recorded minimum (10.33 cm) in and PBW-343. However original internodal distance was found affected incase sown delayed i.e., 18cm and 7.5cm. The percent reduction in internodal distance is quite random. In case of Ist internode the reduction was maximum in UP-2113 (37.14 %) followed by PBW-175 (34.37%). While it was minimum in VL-616 (7.69%) followed by VL-421 (16%). The % reduction in IInd internodal distance, was also found to be maximum in UP-2113 (34.4%) followed by PBW-175(20%) while minimum reduction was recorded in VL-421 (8.35%) , PBW-175 (20%), VL-616 (11.30%) and UP-2338 (2.37%). Their analysis of variance clearly shows that timely sown cultivars have significant higher value as compare to delayed sown (App VI D)

4.2 CHLOROPHYLL FLUORESCENCE VARIABLE YIELD OF DIFFERENT WHEAT GENOTYPES

The photosynthetic efficiency of various leaves in all wheat genotypes has been monitored using Plant Efficiency Analyzer (Handy PEA). The observations have shown that the flag leaf reflected superior efficiency compared to all other remaining leaves. Therefore, it was chosen as a model leaf throughout experimentation. Apart from this, Fv/Fmax values were also monitored using ear head i.e., spike

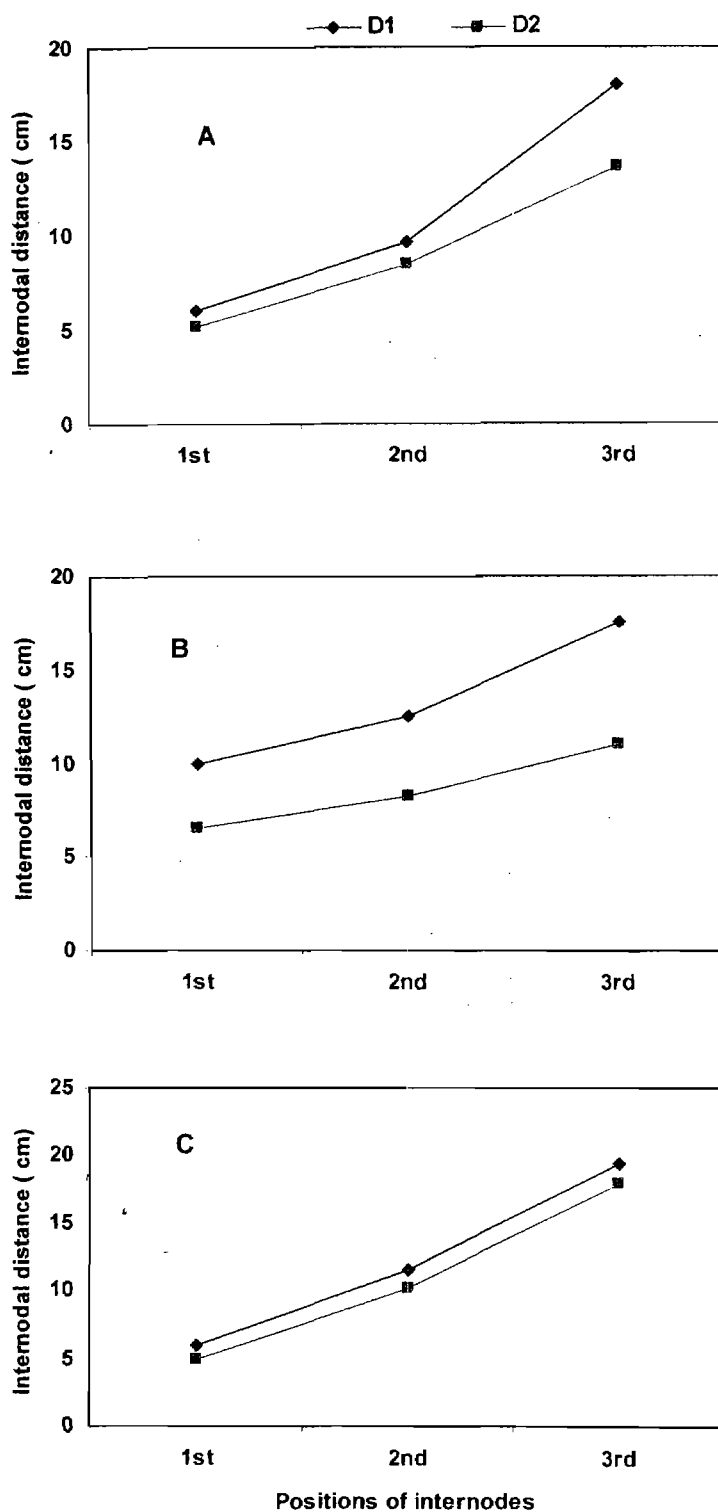


Fig 4.4 Effect of date of sowing schudles on the acquisition of internodal distance in different wheat cultivars. A: UP-2338, B: UP-2113 and C: VL-616

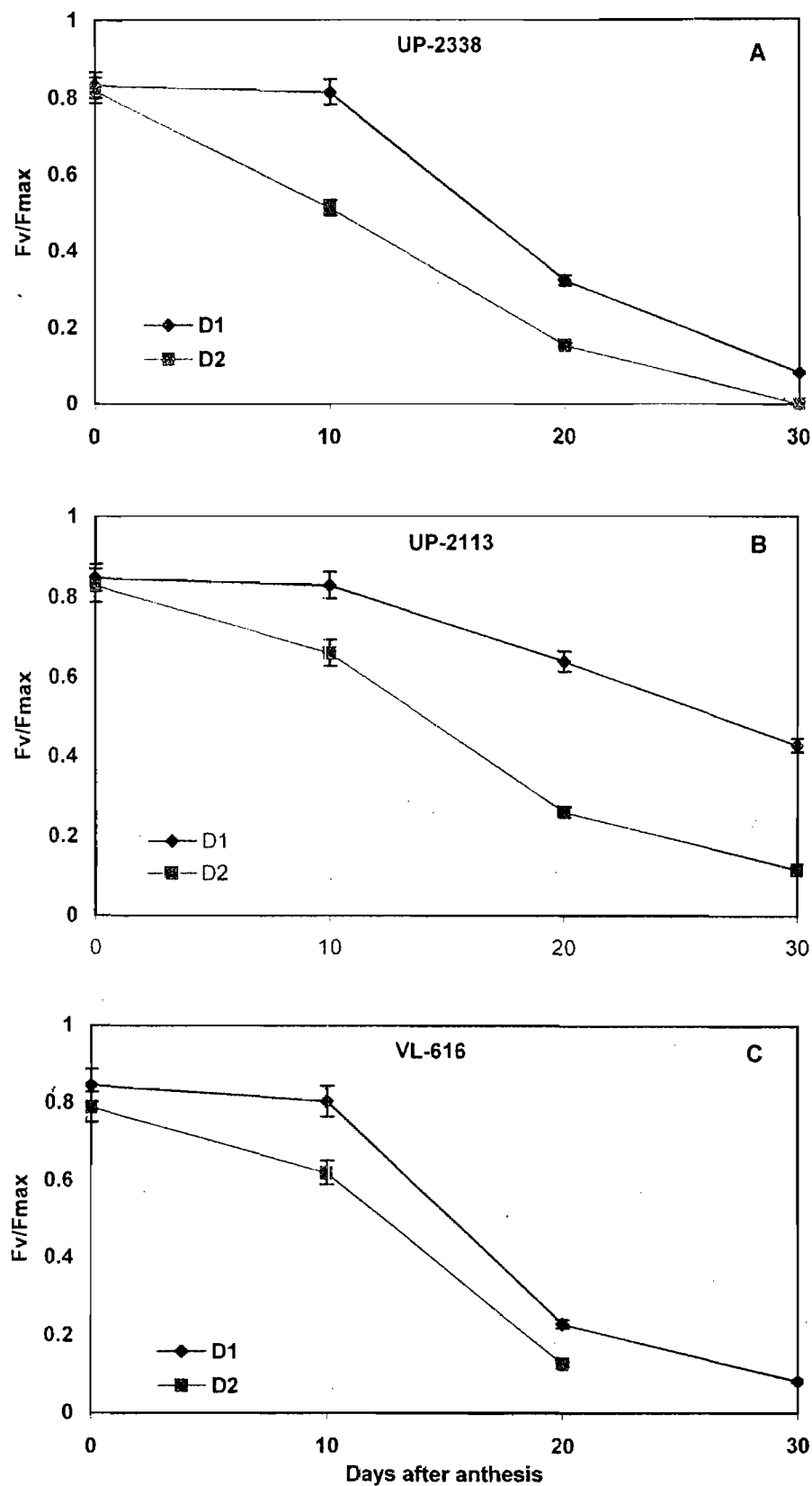


Fig. 4.5: The chlorophyll fluorescence variable yield (F_v/F_{max}) of flag leaf monitored after anthesis under time and delayed sown conditions Data are mean $\pm$ SE of five independent measurements.

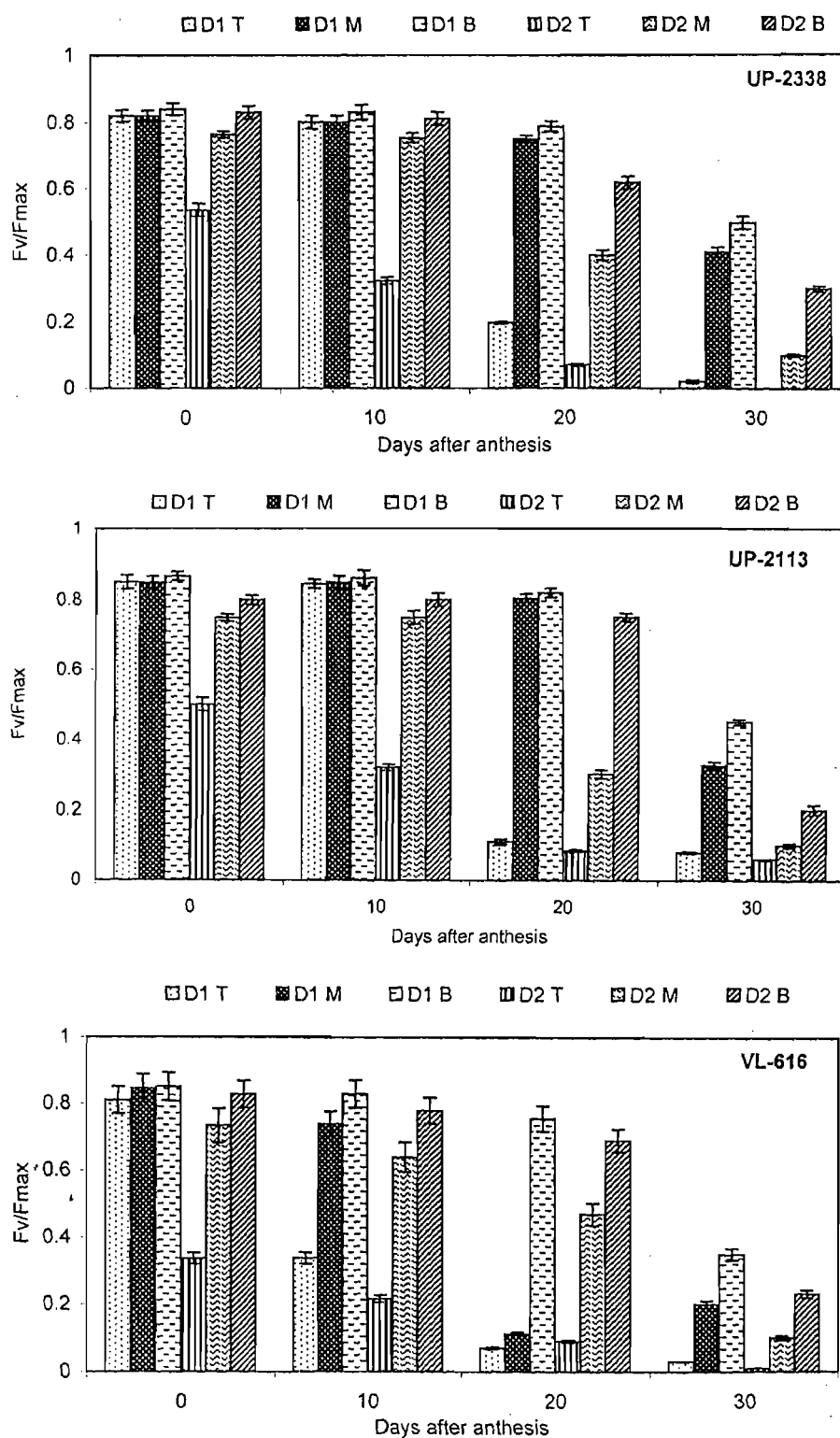


Fig: 4.6 Expression behaviour of chlorophyll fluorescence variable yields (F_v/F_{max}) shown by basal (B), middle (M) and tip (T) segments of the flag leaf. D1 and D2 indicate two different sowing schedules (time and delayed sown).

and also in different segment of the flag leaf i.e. (tip, mid and base) to evaluate Chlorophyll Fluorescence Variable Yield trends opted by various segments/ parts of wheat plant(Fig 4.5 , 4.6 and 4.13). The chlorophyll fluorescence variable yield was always higher in case cultivars sown timely, shown by various flag leaf segments and ear head (App IIA, B, C). The tip segment of flag leaf has been found an indication to observe the loss in F_v/F_{max} values parallel to the days after anthesis. The cultivar VL-421 (App IIA) has been most susceptible in losing F_v/F_{max} values along with maturity. The trend was also followed by other cultivars less intensively particularly by UP-2113 and UP-2338 and VL-616 (Fig. 4.5). The higher F_v/F_{max} values just after anthesis shows better performance of PSII, which starts getting down regulated to retain its ability in case maturity continues (App VII A, B).

4.3 PHOTOSYNTHETIC PIGMENTS (CHLOROPHYLL CONTENT)

The loss of chlorophyll is the most visible event during leaf senescence so, in the present study, chl (a+b) and chl a/b ratio was monitored after anthesis in different genotypes sown timely and delayed. The loss in chl (a+b) content was almost unaffected till 10 days after anthesis (Fig 4.7 A, B, C). However, afterwards i.e., 20 and 30 days after anthesis has shown loss in chlorophyll pigments ca 25-30% . The trend of loss in chlorophyll and chl a/b ratio was much sharp in the cultivars sown delayed . Indeed, the basal values of chlorophyll pigment and the chl a/b ratio was always superior i.e., higher compared to the delayed sown wheat cultivars. The higher values of chlorophyll was found ca 4mg/gm fw along with chl a/b ratio 3.9 (App II C) in UP-2338 if sown timely (D1) while it reduced up to 1.5 and 1.6 respectively in delayed sown conditions(D2).

4.4 ANTIOXIDANT ENZYMES

4.4.1 Superoxide dismutase (SOD) and peroxidase activity

SOD and APX enzymes were studied mainly in three varieties viz. UP-2338, UP-2113 and VL-616 sown in time and delayed as

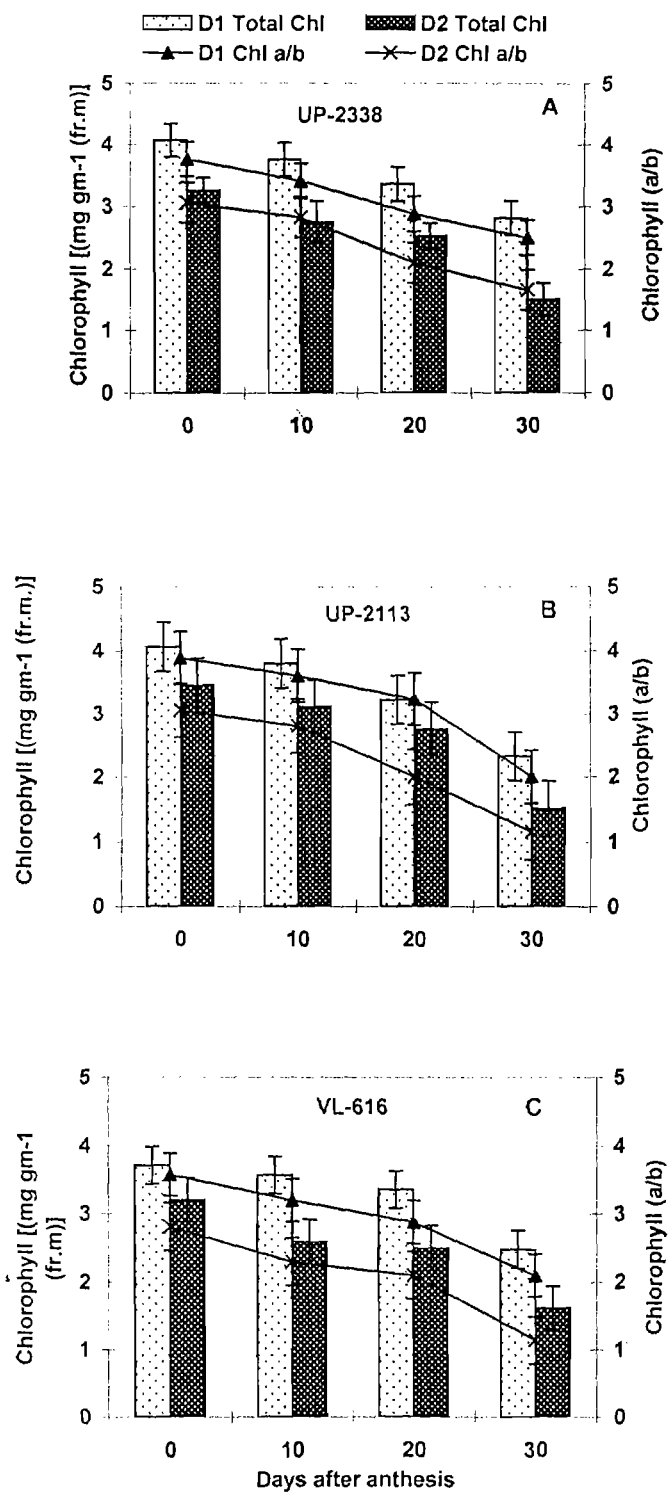


Fig. 4.7: Changes in photosynthetic pigments chl (a+b) content and chl a/b ratio shown by field-grown wheat cultivars in each case data are mean $\pm$ SE of five independent measurements.

abbreviated by D1 and D2. Fig 4.8 A, B and C indicate the up-regulation of stress inducible enzymes viz., superoxide dismutase and peroxidase along with the duration after anthesis. Initially, these values were found almost 20-25% compared to the values being observed after 30 days of anthesis. Actually, higher the anthesis duration get co-related well with the light, temperature and humidity stress along with dry air. All these abiotic stresses switch-on biosynthesis of these enzymes which try to mitigate the adverse environmental variables to the great extent. Since, timely sown cultivars enjoyed higher number of days for maturity, therefore they have higher acquisition of SOD and APX at 30 days after anthesis compared to timely sown same cultivars which did get mature quickly with lesser enzyme activity/ protein of these enzymes (App II D, E) .

4.5 MELANODEALDEHYDE CONTENT (MDA): LIPID PEROXIDATION OF MEMBRANES

The flag leaf was taken at different time interval from both timely sown (D1) and delayed sown (D2) using varieties viz. UP-2338, VL-616 and UP-2113 (Fig 4.9 A, B, C) .

In all the varieties increment in MDA content was found nearly parallel with the crop maturity or reaching towards senescence. The enhancement of MDA content was found almost four folded in delayed sown cultivars compare to timely sown. Apart from this, even timely sown cultivars have shown significant ca more than two fold up-regulation of MDA content after the on-set of anthesis, while delayed sown cultivars opted enhancement in the MDA content to the tune of almost 3-4 folds. These trends were found very much similar and faster in UP-2338 and VL-616, while UP-2113 opted rather gradual change in acquiring enhancement of MDA content (App II F).

4.6 ULTRA STRUCTURAL CHANGES IN CHLOROPLAST STRUCTURE

Chloroplast are one of the earliest sites of catabolism during leaf senescence. The photosynthetic apparatus localized in chloroplast is

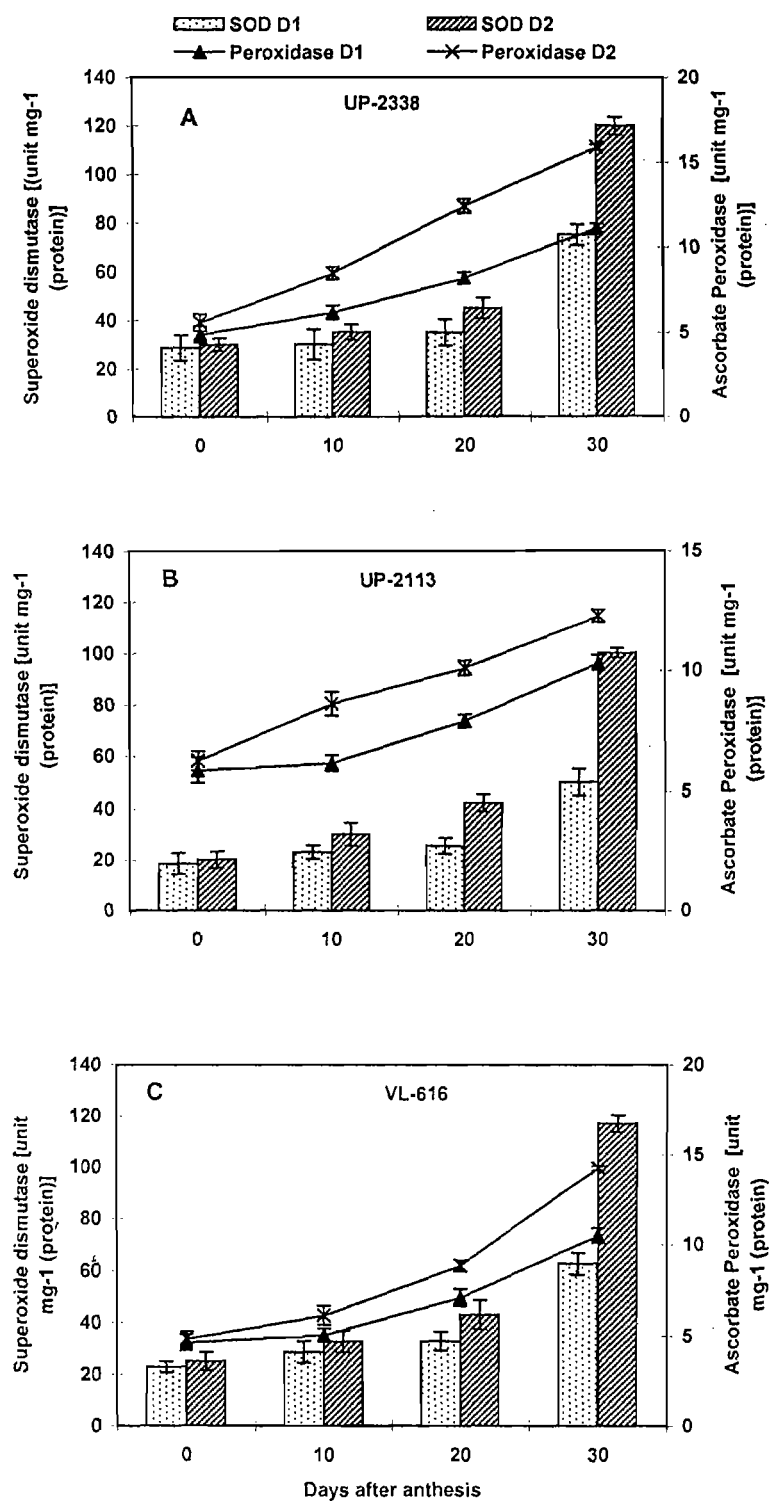


Fig. 4.8: Peroxidase and superoxidase dismutase enzymes activity in various wheat cultivars sown in time (D1) and delayed (D2). The data represent mean values of at least three independent measurements

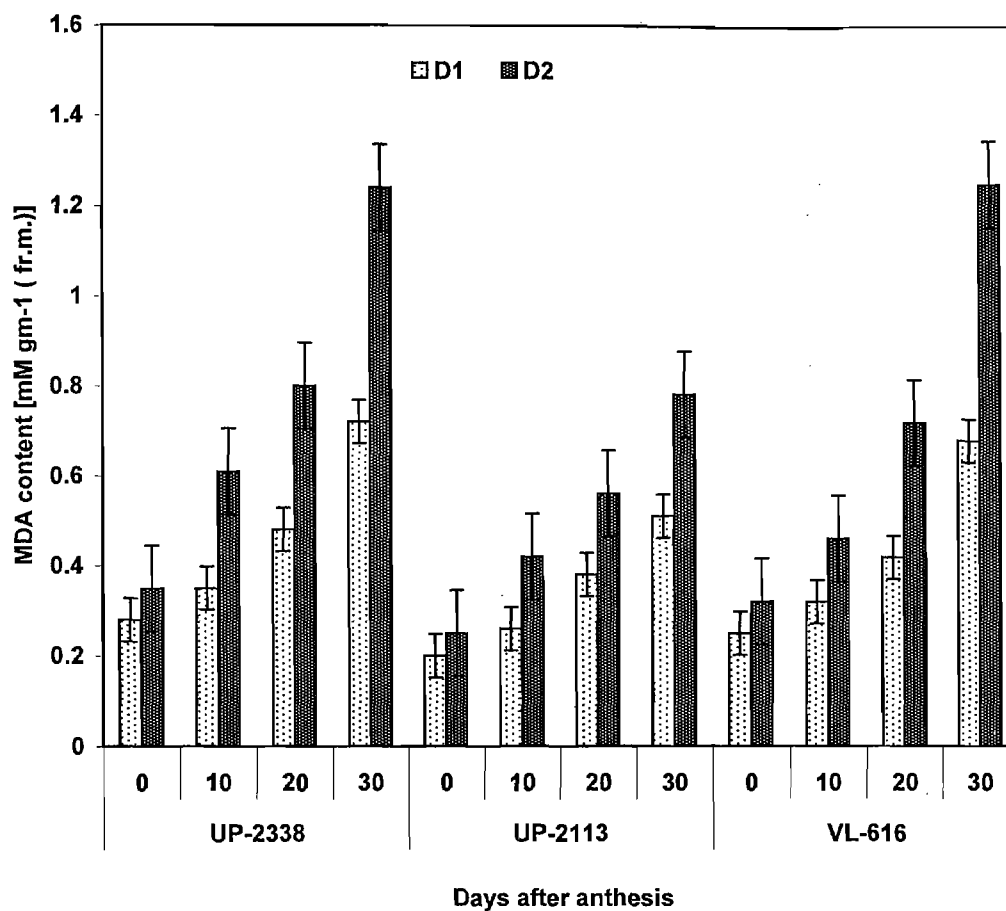


Fig. 4.9: Peroxidation of thylakoid lipids on photoinhibition of different cultivars of wheat flag leaf at different time intervals, sown in time (D1) and delayed (D2). The data represent mean $\pm$ SE of at least three independent measurements

UP-2338 (D1)- timely sown crop

Fig : 4.10 (A) : Normal plastid morphology of green leaf **(B) :** enlarged view of normal plastid **(C) :** rapidly deteriorating and **(D):** Completely dismantled photosynthetic organization of the plastid / chloroplast

UP-2338 (D2)- delayed sown crop

Fig : 4.11 (A) : Normal plastid morphology of green leaf (B) : enlarged view of normal plastid (C) : rapidly deteriorating and (D): Completely dismantled photosynthetic organization of the plastid / chloroplast

the most sensitive structure to various stress factors i.e., environmental and anthropogenic. The changes in chloroplast during the onset of senescence revealed through electron microscope indicates that the stroma and grana thylakoids integrity is highly regulated in the adverse environmental variables. In the present study, variety-2338 (both at D1 and D2 dates of sowing) was chosen to monitor the consequences of on-set of senescence on photosynthetic thylakoid membranes. The electron micrographs indicate about the normal morphology and ultra structure of the chloroplast (Fig 4.10 and 4.11). However, the normal integrity on slackness of chloroplast get impaired due to senescence. The impairment gets continued with the severity of senescence (Fig 4.10 C and D). The dismantling of grana lamellae is very well being observed caused by senescence, associated with morphological appearance of leaves and gradual loss in photosynthetic ability of the wheat flag leaves. Indeed, wheat flag leaves experiencing elevated temperature, sharp irradiance and dry air induced morphological and ultra structural changes. Thylakoids membrane get swollen and slightly disoriented during the first phase of senescence, followed by disorganization of the stroma and thylakoid membrane. These symptoms were more prominent / earlier in delayed sown conditions (D2) in the same genotype i.e., UP-2338 (Fig. 4.11C, D).

4.7 BEHAVIOUR OF FLAG LEAF SENESCENCE

In the present study, flag leaf is considered to be the experimentally potent material, as it is the leaf which is late to come and late to go. Thus it lives longer with maximum its all higher contribution for photo transformation of light energy and also to extend long term photosynthate sink to the developing grains during anthesis and maturity as well. Data given in the Fig 4.12 A, B, C and App. III A clearly shows that flag leaf gets more quickly senescence under D2 as compared to D1 sowing schedule. The percent

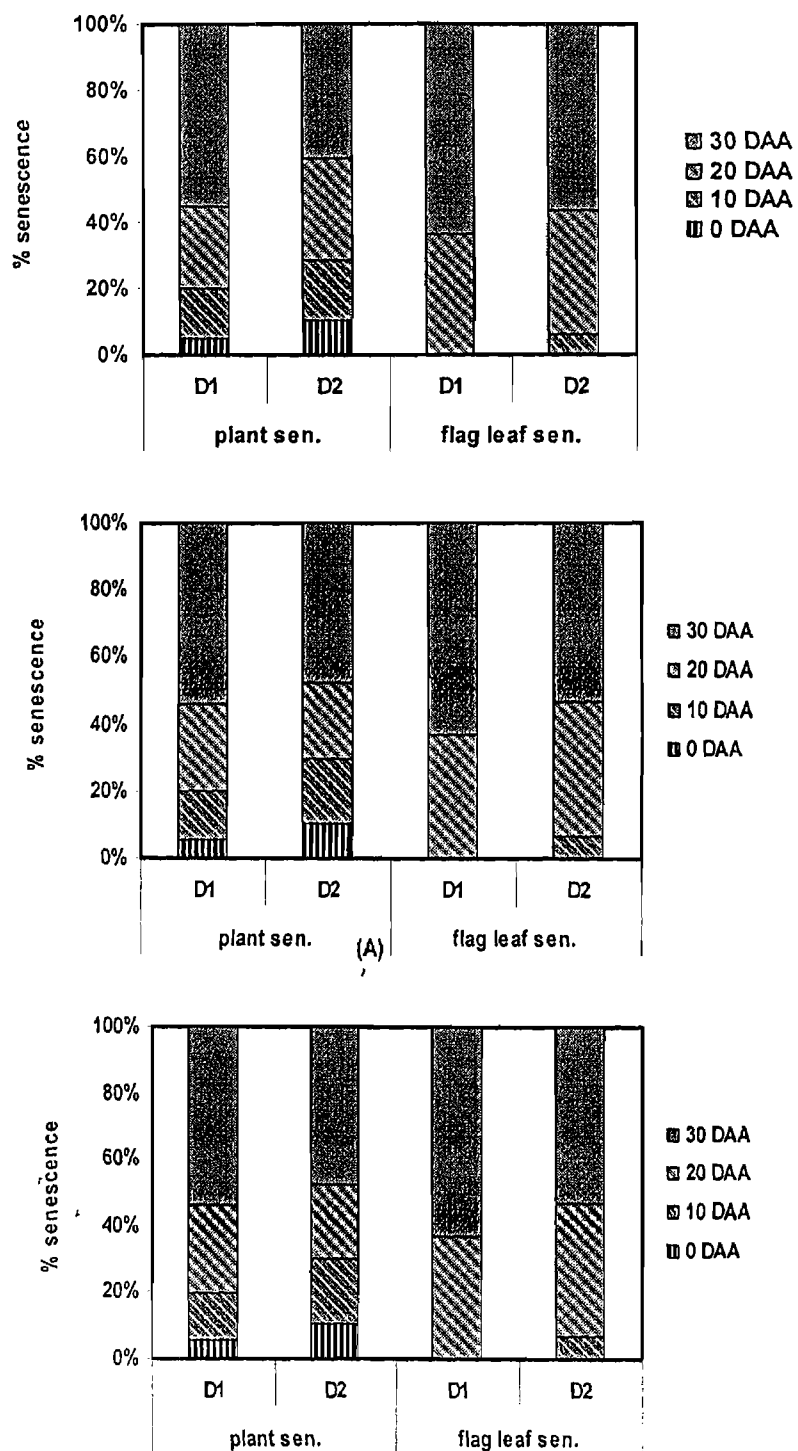


Fig: 4.12 Effect of date of sowing schedules (D1 and D2 i.e. timely and delayed sown) wheat cultivars. A: UP-2338, B: UP-2113 and C: VL-616

senescence was always found significantly higher in D2 as compare to D1 (App VIII A).

4.7.1 Behaviour of Whole Plant Senescence

Fig 4.12 A, B and C indicate complete senescence of flag leaves after 30 days anthesis in all cultivars (UP-2338, UP-2113 and VL-616). However, all the cultivars represented ca 40% senescence after 20 days after anthesis compared to the time sown cultivars which represented the senescence always less than 40%(ca35%) (App. III B), while time sown cultivars did not show flag leaf senescence during the anthesis. However, time and delayed sown cultivars represented ca 5-10% total plant senescence during anthesis in each cultivars (App. VIII B).

4.7.2 Ear/Head Percentage senescence (Maturity)

The ear-Head maturity was observed at 10, 20 and 30 days after anthesis, having two dates of sowing abbreviated as D1 and D2 (Fig 4.13 A, B, C and App III C) which indicates that the ear-head also contributed for photosynthesis equally similar to the flag leaf. The ear-head was found almost equal to flag leaf F_v/F_{max} performance (ca.0.8). Initially, D1 and D2 schedules have expressed similar optimum potential in relation to PSII photosynthetic ability. The ear-head maturity impaired the photosynthetic performance from ca. 50% and ca.80%, incase wheat cultivars i.e., UP-2338 sown in time and delayed sown conditions. The similar trends with severe loss in F_v/F_{max} were also being represented by VL-616 and UP-2113 (Fig 4.13 A, B and C). The percent ear-head senescence/ maturity starts 10days after anthesis (ca.5-20%) , which kept on enhancing (20-45%) followed by ca.80% and complete senescence on maturity after 30days after anthesis, incase sown under D1 and D2 sowing schedules (App XIII C).

4.8 LIFE SPAN

The total life span of a plant depends upon two phases i.e., vegetative followed by reproductive. Both the phases are important to

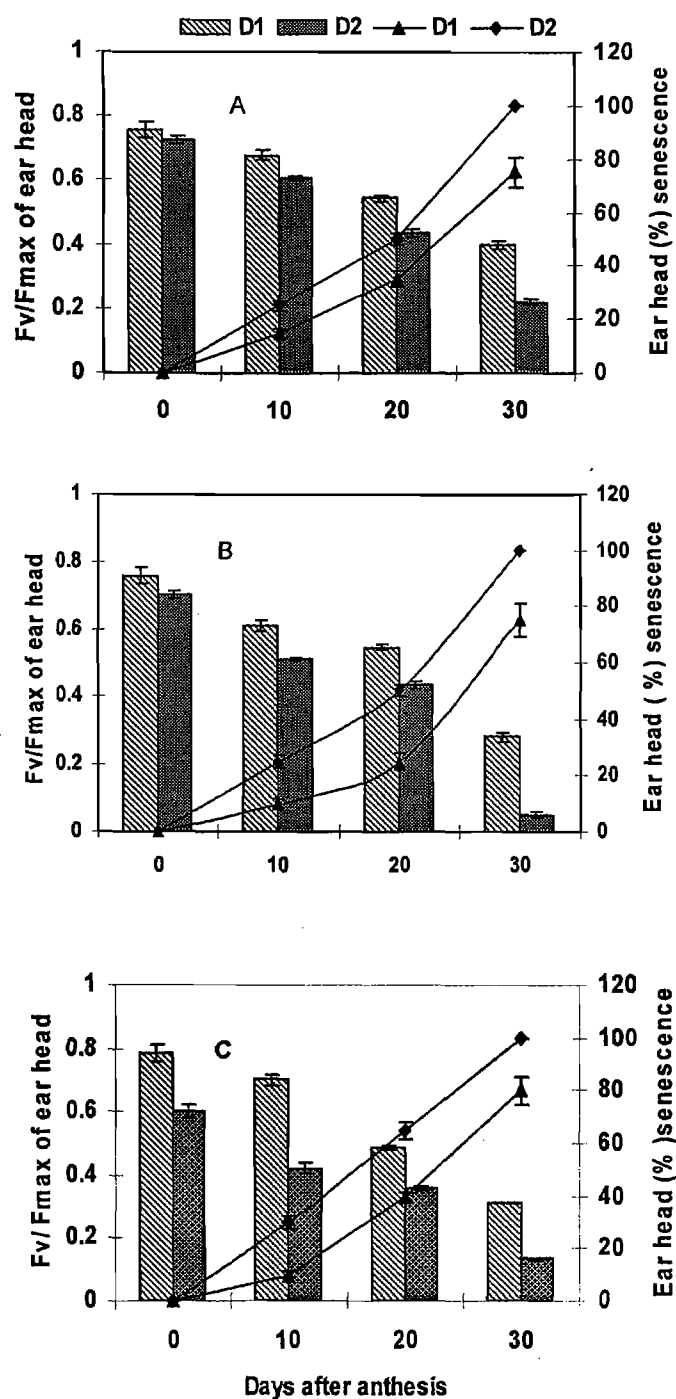
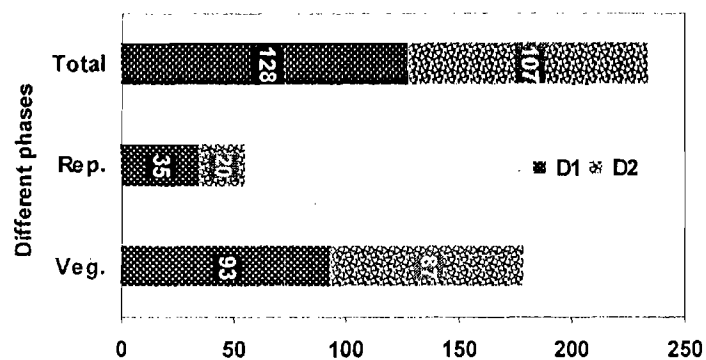
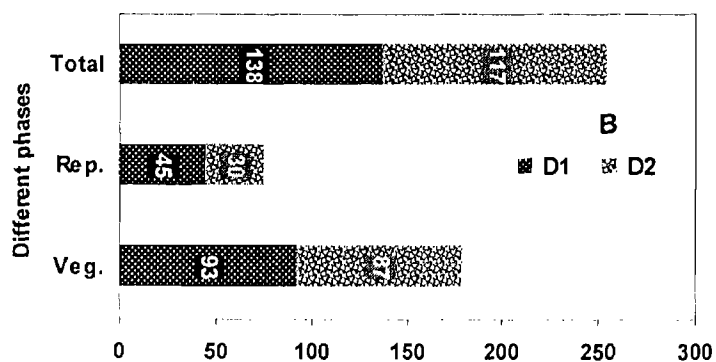


Fig: 4.13 Effect of date of sowing on % ear head senescence and their corresponding PSII efficiency (F_v/F_{max}) in (A) UP-2338, (B) UP-2113 and (C) VL-616

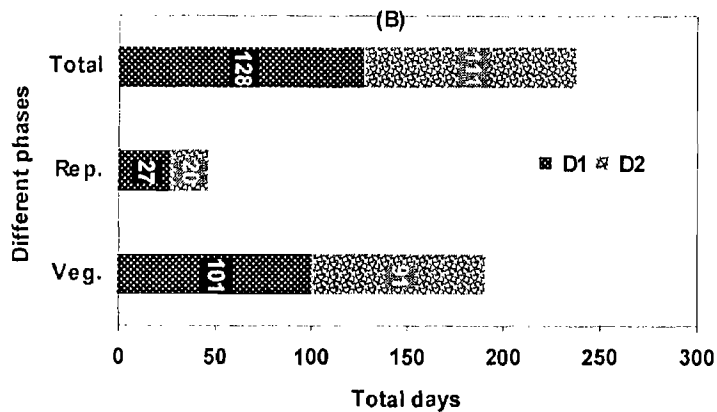


(A)



B

(B)



Total days

(C)

Fig: 4.14 Effect of date of sowing on vegetative, reproductive and total time required to complete its life cycle
: (A) UP-2338, (B) UP-2113 and (C) VL-616

determine several physiological characteristics of a plant. On the basis of normal and abnormal life span shown by wheat cultivars, one can easily correlate or predict about the resistance and sensitiveness of a plant getting experienced by either favorable or adverse environmental variables. The data shown in Fig. 4.14 A, B, and C (also App IV D) indicate that the days taken by vegetative phase were always higher than the maturity completion. Indeed, the similar trend was seen with all wheat cultivars. Consequently, total days taken for reproductive phase were 27 and 22% lower in case of VL-616 followed by ca. 50 and 34%, 37 and 23% in case of UP-2113 and UP-2338 respectively. The delayed sown all cultivars enhanced maturity which took lesser time span due to getting exposed under adverse environmental variable compared to time sown cultivars (App IX A).

4.9 PROTEIN PROFILING THROUGH SDS-PAGE AND WESTERN - BLOT ANALYSIS

The data shown in Fig 4.15 indicates about the SDS-PAGE profile expressed by the flag leaf under timely and delayed sown condition, different segments of flag leaf and grain proteins at different intervals (Fig 4.16) using a wheat variety i.e. UP-2338. The protein profile indicates about the prominent appearance of a band having almost 55KDa molecular size, specifically expressed for Rubisco LSU. Apart from this, several bands seen during anthesis which get either disappeared or diffused, associated with the maturity schedule, this degradation was more prominent in delayed sown condition. Apart from (rbc L), the degradation of (D1) protein is also clear as crop attains maturity or get senescent. Besides it, these profile confirms the onset of senescence from the upper part (tip) of a flag leaf reaching towards base. Western- Blot data of Rubisco favours that LSU encoded by rbcL of plastid genome gets expressed maximally during anthesis. However, its expression gets down-regulated with maturity/ senescence (Fig 4.17).

Fig 4.15 SDS – PAGE profile of total soluble flag leaf proteins stained with coomassive brilliant blue (R- 250). Each lane was loaded using 100 μ l sample size ($\approx$ 12.5 mg of protein) .

Lane M: indicates Molecular marker
Lane 1: sample harvested during anthesis (D1)
Lane 2: 10 days after anthesis (D1)
Lane 3: 20 days after anthesis (D1)
Lane 4: sample harvested during anthesis (D2)
Lane 5: 10 days after anthesis (D2)
Lane 6: 20 days after anthesis (D2)

D1 and D2 indicates timely and delayed sown crop

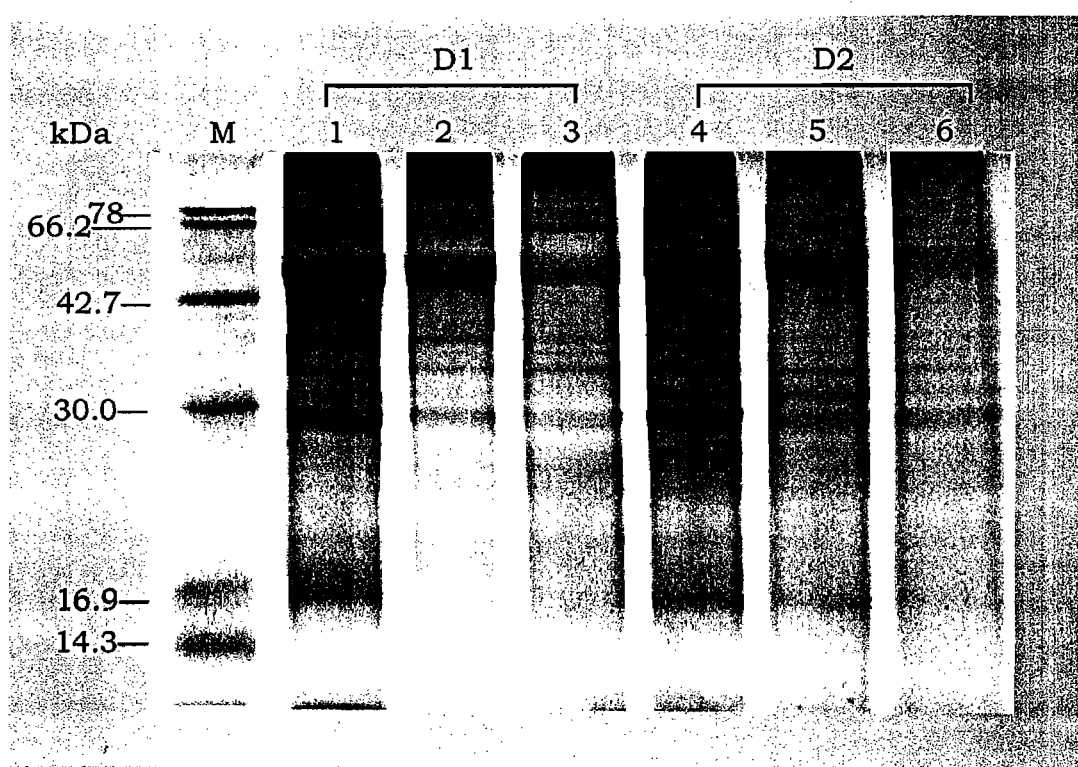


Fig 4.16 SDS – PAGE profile of total soluble flag leaf and grain proteins silver stained . Each lane was loaded using 50µl sample size ($\approx$ 6.25 mg of protein) .

Lane M: indicates Molecular marker
Lane 1: sample harvested from tip part
Lane 2: sample harvested from mid part
Lane 3: sample harvested from base part
Lane 4: sample (grain) harvested during anthesis
Lane 5: sample (grain) harvested 15 days after anthesis
Lane 6: sample (grain) harvested 30 days after anthesis

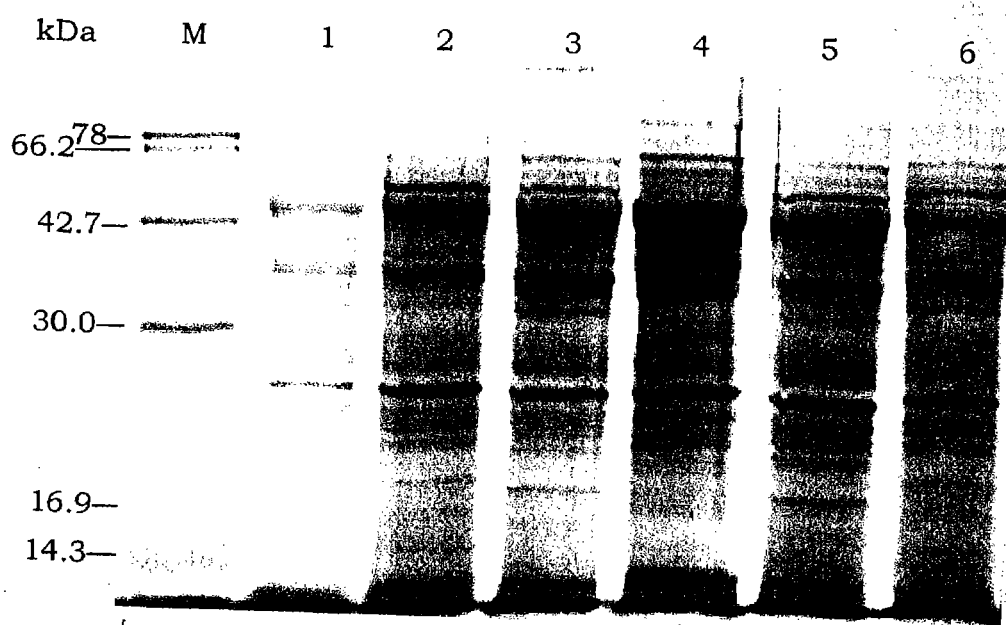
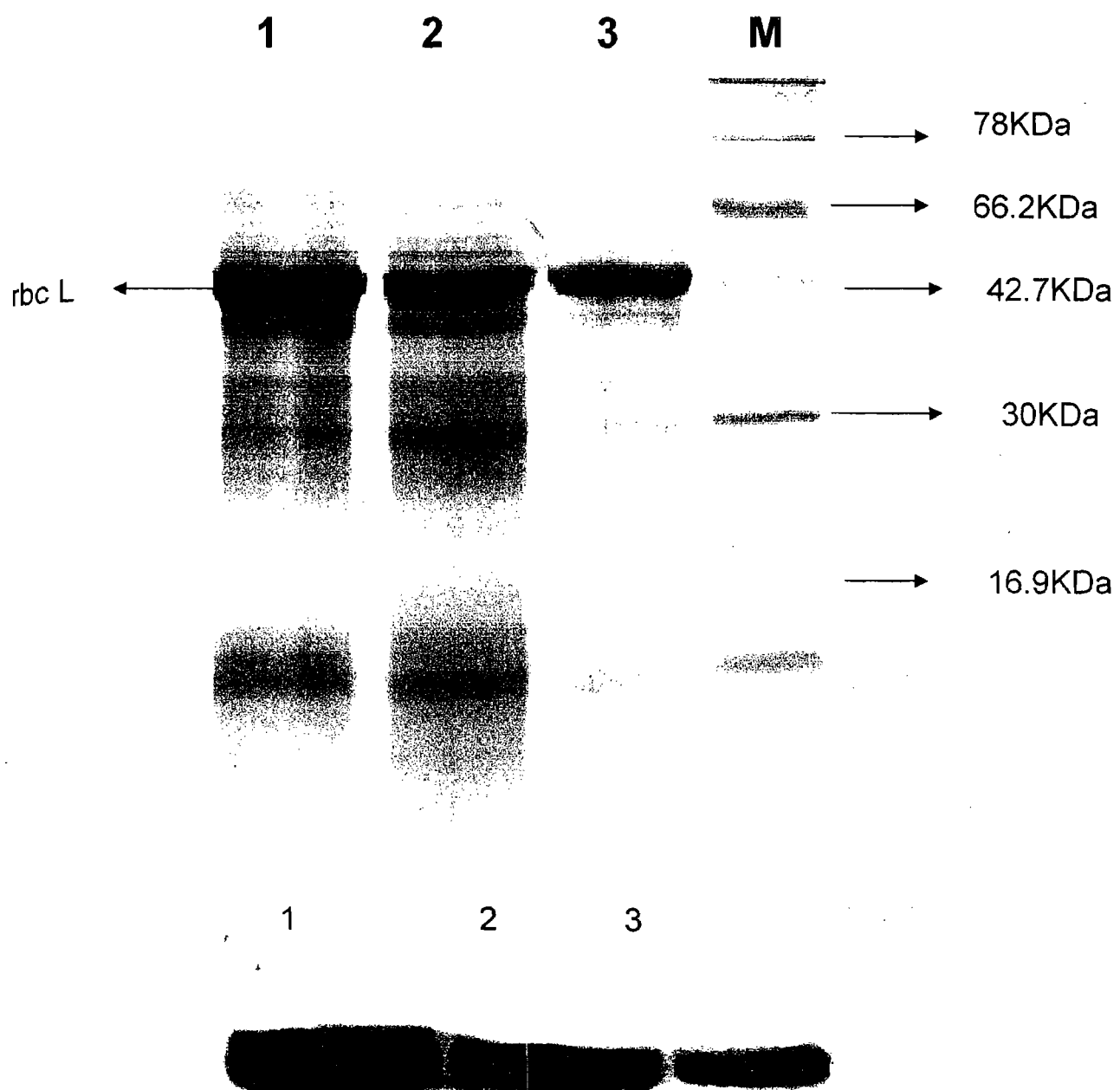


Fig 4.17 A SDS – PAGE profile of total soluble flag leaf proteins stained with coomassive brilliant blue (R- 250). Each lane was loaded using 100µl sample size (≈ 12.5 mg of protein) .

Lane 1: sample harvested during anthesis
Lane 2: 10 days after anthesis
Lane 3: 20 days after anthesis and
Lane M: indicates Molecular marker

Fig 4.17 B Western – blot analysis of Rubisco LSU. Rubisco LSU was detected using specific anti- rubisco antibody.

Lane 1, 2 and 3 represent Rubisco LSU detected at the on-set of anthesis, 10 and 20 days after anthesis respectively.



4.9 YIELD ATTRIBUTES

After the completion of life span / senescence / maturity, plants were harvested and their yield attributes were recorded in terms of 1000-grain weight, grain yield and harvest index in both D1 and D2. In all the above three parameters, it is clear from Fig 4.18 A, B and C as well as App. V A, B and C that date of sowing has significantly affected these yield parameters in negative direction.

The value of 1000-grain weight was found to be maximum in UP-2425 (45gms), and is significantly higher as compare to the rest of the varieties except PBW-343 (App XC) . On the other hand, PBW-175, is showing the minimum value i.e. 27.96gms (App. V C) . In terms of percentage reduction in 1000-grain weight, it was found to be maximum in VL-421 (19.23%) followed by HD-2687 (16.24%), while min. reduction was seen in UP-2113 (5.73%).

The value of grain yield was found to be higher in UP-2113, and was significantly higher to only VL-616 and PBW-175 (X B).Percentage reduction in grain yield, does not show much variation, amongst different genotypes; but still maximum % reduction was shown by VL-421 (38.57%) followed byPBW-175(36.11%).The value of harvest index was found to be highest in UP-2338, but this difference was not significantly higher from the rest of the varieties (XA).

Percentage reduction in harvest index was found to be maximum in PBW-175 (44.29%), while it is minimum in UP-2338 (31.8%) . In other genotypes, this reduction was (41.21%) in VL-421, UP-2113(32%) , PBW-343 (35.64), VL-616(38.37%) , VL-804 (38.60%) and 39.5% in UP-2425 (App. V D).

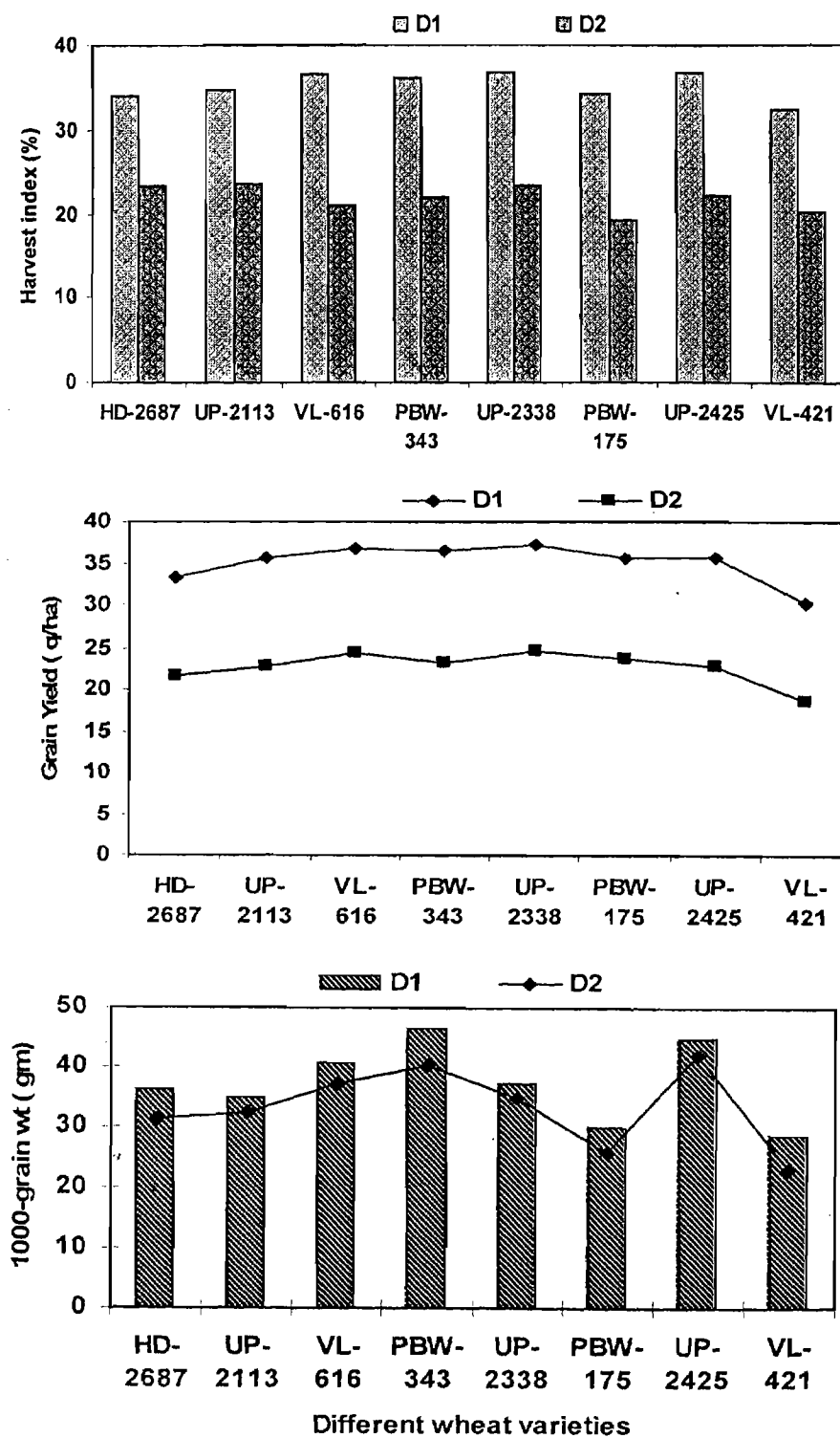


Fig: 4.18 Effect of date of sowing schedules on % Harvest index, grain yield and 1000-grain weight in different wheat cultivars

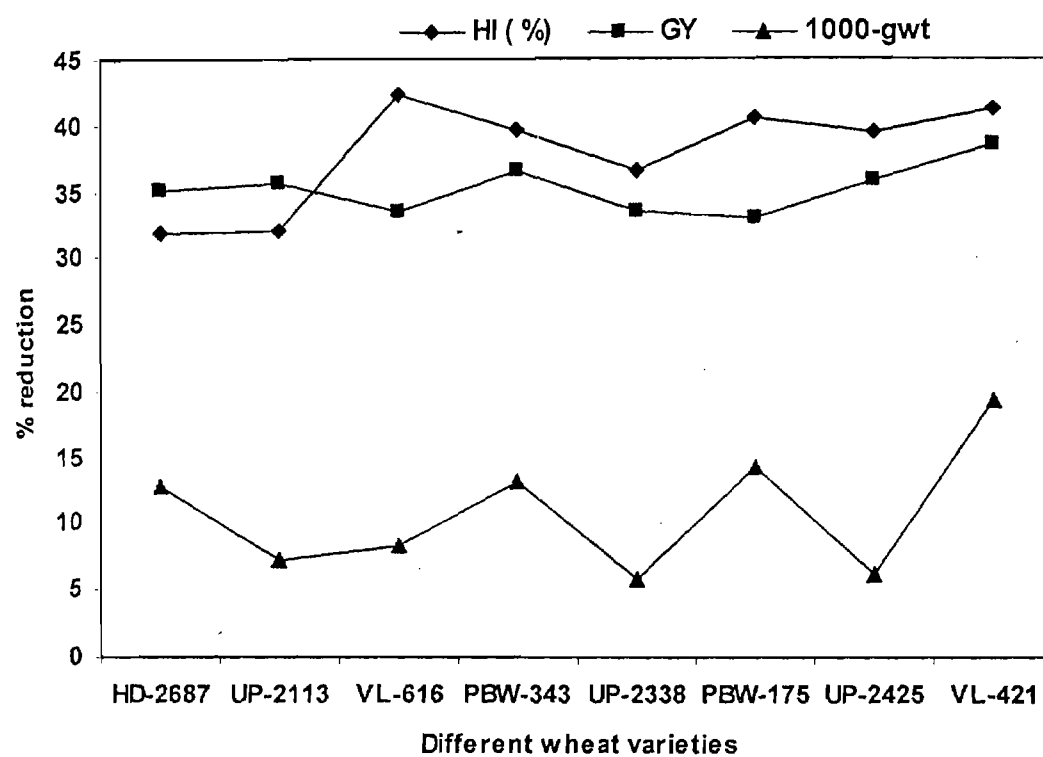


Fig 4.19 Showing percent reduction in different yield attributes viz. % Harvest index, grain Yield and 1000- grain weight in different wheat cultivars



Discussion.....

Plants are often exposed to high temperature and irradiance stress under field conditions, which usually create water deficit in plant tissue. The consequences of these factors have already been studied in ecophysiology and cell metabolism (**Bose and Ghosh, 1995; Shinozaki and Yamaguchi, 1997; Dekov et al ., 2000**). Indeed , the importance of changes due to these stress characteristics depend on the plant capacity and sensitivity, as well as on the intensity and duration of the stress, either applied alone or in combination (**Bhadula et al., 1998**). The negative effect of high temperature is likely to occur more in those crops which are adapted to temperate climates such as wheat, barley, cauliflower etc. as their thermal requirement for optimum growth and productivity is less as compared to other crops (**Watson, 1995; Singh et al 2001**). The sudden rise in temperature enhances forced maturity / senescence because it hastens the phenological development of the crop which reduces the grain filling period , eventually reducing grain yield (**Wang et al ., 1992 ; Yang et al., 2001**).

Senescence is an essential developmental process associated with plants which eventually leads to either whole plant or its parts viz. organ, tissue etc. followed by cell death through highly regulated, genetically controlled processes (**Quirino et al ., 2000 ; Chandlee, 2001**). Leaf senescence is a key developmental state in the life of a plant, and is characterized by specific cell ultrastructural changes (**Buchanan et al ; 2000**) and by chlorophyll loss (leaf yellowing), oxidative stress (**Bosch and Penuelas , 2003; and Spano et al., 2003; Upadhaya and Panda, 2004**) and decrease in photosynthesis (**Jiao et al., 2003 ; EL- Shintinaway et al., 2004**) . The sowing schedule has affected shoot length and spike length (Figs 4.1 and 4.2). Indeed, delayed sown cultivars minimized their shoot length and spike length due to imposition of adverse environmental variables i.e. high temperature, dry air coupled with sharp irradiance and loss in

humidity . The similar findings or trends have already been reported (**Xi-ping *et al.*, 2000**)

Efficient translocation of photoassimilates to growing seeds is one of the key factor to increase seed production (**Yamagata *et al.*, 1987; Aldesuquy, 2000**) . The main source of carbon for grain filling in wheat is that produced by current photosynthesis through flag leaf (**Austin and Edrich 1975; Wenshan *et al.* , 1994**). When $^{14}\text{CO}_2$ is fed to flag leaf of an intact plant, only a minor proportion of the ^{14}C fixed is transferred to part of tillers, however the major proportion of photosynthate is transported to spike (**Wenshan *et al.*, 1994**). The importance of leaf area in controlling plant growth and productivity was confirmed by **Potter and Jones (1977)** who concluded that the rate of leaf area expansion have a greater influence on dry matter production . Thus, the flag leaf in cereal plants is a powerful source of assimilates translocated to developing grains. The increase in flag leaf area in the first phase of development is attributed to expansion through water uptake as confirmed by the strong correlation between area and fresh matter (**Viana and Metivier, 1980; Aldesuquy, 1990**). In our case, increase in leaf area seems to be positively correlated with the timely sown schedules . The delayed sown cultivars have followed the similar trend with the loss of total leaf area having various leaf positions (Fig.4.3). The declining trend of leaf area has also been associated with the yield. Generally, flag leaf loses chlorophylls during senescence as well as photosynthetic activity , saccharides and soluble proteins with the consequence of decrease in metabolic activity. **Nooden and Leopold, 1988** found that auxin alters senescence – related processes such as Chlorophyll loss, RNA synthesis and degradation, protein synthesis and degradation , wilting, membrane breakdown and changes in the contents of several enzymes (some increase , some decrease), in ways that are consistent with the senescence- delaying effect.

The maintenance of internodal distance is associated with the plant height The data shown in Fig.4.4 indicates differential loss for

the internodal length. The first internodal distance seems to be more affected in case sown delayed. However, in contrast to the observations reported here, increase in the internodal length has been reported by **Singh and Singh, 2001**.

Chloroplast are one of the first organelles to be targeted by senescence, while nuclei and mitochondria maintain their integrity until the late stages of senescence (**Smart, 1994; Buchanan et al ;2000**). Thus functionality of the chloroplast has been evaluated through Chlorophyll fluorescence variable yield based on the potential contribution of PSII. This includes photolysis of water , liberation of electrons , protons and also eventual operation of photosynthetic electron transport linked with CO₂ assimilation and energy production. Therefore chlorophyll fluorescence yield (F_v/F_{max}) of photosystem II was monitored with the view to access contribution of PSII present in all green leaves individually (Fig4. 5). The trend obtained with different wheat cultivars clearly favors significant importance of the flag leaf irrespective of date of sowing. This was also monitored in different segment of flag leaf starting from top to base (Fig4.6) along with ear head , just to know relationship between PSII efficiency with senescence (Fig 4.13). So far nothing has been reported to the best of our knowledge about differential chlorophyll fluorescence variability (F_v/F_{max}) of a flag leaf segmented as basal, middle and upper leaf parts. Similarly, ear head F_v/F_{max} contribution also has not been revealed yet. Thus , our data favours potentiality of the chlorophyll fluorescence gets downregulated in the flag leaf starting from the upper segment to the lower leaf segments. The similar trend of senescence has also been observed which down -regulated the PSII photosynthetic ability as seen with the flag leaf and ear head both . The loss of photosynthetic performance, due to wheat genotypes exposed to elevated temperature is the direct result of heat on chloroplast functions and vulnerability of photosynthesis. The primary event in thermal inhibition seems to be water splitting/ O₂ evolving system of photosystem II reaction centre

(**Vass et al., 1992**). It has also been recorded that heat shock impairs the degradation of D1 and D2 proteins of the photo system II reaction centre core proteins (**Maria et al., 1997 ; Trebst et al., 2002**). Besides it, chlorophyll fluorescence parameters are good indexes for the assessment of the physiological state of PSII (**Singh et al., 2003 ; data under publication**). The decline in F_v/F_{max} , shows the enhancement of F_0 which reflects the damage associated with the PSII reaction centre. F_v/F_{max} was consistent with normal chlorophyll content prior senescence while it started declining during the senescence, in case the value reduced with flag leaf under natural conditions. The down-regulation of PSII photochemical efficiency and photon energy transport efficiency may be one of the intrinsic physiological features of flag leaf during senescence (**Jiao et al., 2003**).

It has been reported earlier that the decrease in photochemical efficiency of PSII at low leaf water contents (**Calatayud et al., 2000**) may also be caused by the down regulation of the capacity of PSII electron transport (**Osmond, 1994., Martinez- Carrasco et al., 2002**). Our experimental surroundings were quite favorable in terms of scarce rainfall, high temperature, sharp and dry air to create forced senescence. Most of the decrease in photon energy used for photochemistry can be explained in terms of reduction in the efficiency of electron captured by open PSII centers (**Cornic, 1994**), involving increase in the non - photochemical dissipation of excess excitation energy (**Osmond, 1994**).

Changes in enzyme amount and activity and contents of metabolites of the Calvin cycle (**Kobza and Seemann, 1989**) during the day affect the rate of carbon assimilation and can consequently alter the rate of photosynthetic electron transport and the fraction of absorbed photons that is dissipated non- photochemically (**Martinez- Carrasco et al., 2002; Sayed, 2004**).

The most obvious symptom of leaf senescence is the loss of chlorophyll. Normally apparent leaf yellowing is seen during

senescence due to chlorophyll degradation and also due to retention of carotenoids (**Matile, 1992; Feller and Fischer, 1994; Smart, 1994; He *et al.*, 2002; Bosch and Penuelas, 2003**). In the present study, the highest value of total chlorophyll was found to be about 4mg/gm of fresh weight in UP-2338 and UP-2113 along with Chl a/b ratio, while the lower values were seen in VL-421. The delay in date of sowing has adversely affected the chlorophyll content and chl a/b ratio.

The decrease in chlorophyll levels and in the chl a/b ratio between mid of March till April were the first symptom of senescence observed in chloroplast of wheat flag leaves (Fig 4.7). The environmental conditions during this period were typical of a sub-tropical climate. From the beginning of March to the beginning of April, precipitation was scarce (2.34mm) and this was accompanied by increase in the diurnal photosynthetically active photon flux density (PPFD) ca. 1800 $\mu\text{mol}/\text{m}^2/\text{sec}$. with midday air temperature (T_a) above 30° C coupled with dry air favored to an early maturation or yellowing in delayed sown wheat crop. A decline in the chl (a+b) along with chl a/ b ratio suggests preferential loss of chl a containing proteins closely associated with the PSII and PSI reaction centers rather than loss of light harvesting proteins (**Bosch and Penuelas, 2003**).

This indicates that the loss of chlorophyll was primarily a response to the combination of strong light , high temperature and dry air. Long term light acclimation of the photosynthetic apparatus typically involves a decrease in the light harvesting antenna size of the PSII and PSI in order to reduce energy delivery to the reaction centers . These observations are quite similar as the chlorophyll content obtained by shade and sun plants (**Barth *et al.*, 2001**). This is a kind of adaptation strategy opted by late sown wheat cultivars, which favors reduction in antenna size in case sown delayed. Because, they experienced high temperature and sharp light (**Hidemma *et al.*, 1992; Niyogi ,1999**). The strategy for reducing antenna size also

minimizes photo inhibition of photosynthesis or promotes photoprotection by not interacting with the high light intensity (**Singh et al ., 1996**).

The photo-inhibition usually originates from the excess photons trapped by leaf. When irradiance was lowered, H_2O_2 and O_2 production was effectively delayed, showing that the initial AOS production was not the direct result of water deficiency but the result of excess photons (**Jia et al ., 2002**). Photosynthesis depends on the function of the light harvesting and electron transport systems within the chloroplast which is inundated by the photochemical efficiency in terms of F_v/F_{max} . Loss of photosynthetic competence can result from several processes including the dissociation and degradation of PSII and Rubisco polypeptides and proteins followed by destruction of membranes (**Thomas, 1987; Mohanty, 2003; Spano et al., 2003**). In plant cells, chloroplasts are one of the most important intracellular generators of activated oxygen species (AOS) such as singlet Oxygen ($^1O_2$), Super oxide radicals, hydrogen peroxide and hydroxyl radicals. AOS specially $^1O_2$ and hydroxyl radicals are highly reactive and in the absence of protective mechanism, can produce damage to cell structure and function (**Asada , 1999; Pandey et al., 2003**). Because several enzymatic and non-enzymatic antioxidants are present in chloroplasts to control oxygen toxicity (**Asada, 1999**). The data shown in Fig.4.8 has similarly been observed with various wheat genotypes. The SOD and peroxidase activities have been up-regulated throughout the maturation period of wheat ie. after anthesis. Indeed, the period of anthesis onwards was associated with the gradual enhancement of dry air, less humidity, sharp irradiance and elevated temperature. These adverse environmental variables forced to all wheat cultivars for their survival through the biosynthesis of SOD and peroxidase.

Beside it, lipid per oxidation (POL) is considered to be the best criteria for damage caused by increasing for damage caused by over production of ROS (**Halliwell, 1991; Ray et al ., 2004**). It occurs ,

when (OH) radicals are generated, which attack the unsaturated fatty acid chain of membrane lipids, which can cause membrane leakage and loss of selective permeability (**Saelim and Zwiazek, 2000**). SOD and peroxidase enzyme activities from flag leaf tissue of late sown plants was higher in UP-2338 and VL-616 as compare to UP-2113 (Fig 4,8A, B and C).However, SOD does not provide complete protection of the cell against oxidative stresses, since H_2O_2 gets biosynthesized as a product of its functioning (**Meloni et al., 2003**). Therefore, peroxidase synthesis also gets induced to destroy H_2O_2 during adverse environmental surroundings. However, SOD catalyzes the dismutation of O^2 to H_2O_2 and plays a key role in the quenching of active oxygen (**Agarwal and Pandey, 2003**).

Plant cells try to evolved defense antioxidant mechanisms to combat the danger imposed by the presence of reactive oxygen species (ROS) . (**Landberg and Greger 2002; Meloni et al., 2003**).The delicate balance between ROS production and its processing by antioxidant enzyme are, crucial to minimize oxidative damage to leaves to extend limited acclimation (**Mallick and Mohn, 2000**). In the present study, Melanodealdehyde content is found to be increased as the plant gains maturity followed by senescence with its higher values in late sown crop compared to timely sown (Fig4.9). The enhanced generation of MDA can be attributed to the oxidation of linoleic and linolenic acids as the components of the phospho- and galactolipids participating in the arrangement of the reaction centers of photo systems in chloroplast membranes (**El- Shintiawy, 1999**). MDA is capable to interact with free amino groups of proteins and with phospholipids molecules (**Rakwal et al., 2003**) . In addition, to this it can also initiate ethylene generation in membranes (**Jiang and Zhang, 2002**) to put the cell under forced senescence all these alterations can modulate the properties of both inter and intracellular membranes, resulting in leakage of various ions through membranes (**Saelim and Zwiazek, 2000 ; Dash and Mohanty, 2002**) .

Chloroplasts are one of the earliest sites of catabolism in leaf senescence as the photosynthetic apparatus localized in it, is the most sensitive system of various stress factors i.e. both environmental and anthropogenic. The yellowing seen in senescence chloroplast may be due to faster degradation of chlorophylls than carotenoids. In terms of pigment composition, senescent chloroplast resemble chromoplasts (**Thomson and Whatley, 1980; Smart, 1994**). The chloroplast persists and runs through senescence – specific differentiation. The final structure created by senescence transition is termed as ‘gerontoplast’. Severe stress causes an inhibition of protein synthesis, inactivation and dissociation of some chloroplast enzymes/ proteins; especially those participating in CO₂ assimilation. The regenerative phase of Calvin cycle, electron transport and capability for photophosphorylation also get disturbed and this may be due to the significant changes in chloroplast ultra structure (**Dekov et al., 2000**). The ultra structures of chloroplast viz. intactness of grana thylakoids has been found gradually impaired during on-set of maturity. The stroma lamellae were also found altered. The impairment of these structures favors non-functionality of the PSII and PSI associated with light and dark phases of the photosynthetic process of any green tissue.

The Fig 4.10 and 4. 11 shows structural changes in the thylakoid such as loosening and disorganization of grana stacks, swelling, vesiculation and progressive loss of membranes, this is all due to the transition of Cpls into Gpls (**Matile, 1992; Thomas, 1997**). The stroma components are also lost with the loss of their volume and density. A conspicuous feature of transition is the increase of plastoglobules in number and size, in which undigested or indigestible lipophilic components of thylakoid membrane get accumulated. Eventually, fully differentiated Gpls consist of a still intact envelope wrapping up a large No. of plastoglobules get appear at the end of senescence. Estimation of functional and structural damages of chloroplast during senescence is important for determining the extent

of adaptability and resistance of plant tolerance to senescence acting individually or simultaneously under forced senescence induced by excess of light and elevated temperature, because it happens oftenly in nature (**Dekov et al .,2000, Pechava, 2003 ; Matos et al 2002**).

The profile of percentage senescence of flag leaf and whole plant indicate that delayed sowing may induce senescence quicker than the normal sown conditions, may be due to imposition of adverse environmental variables over late sown cultivars, which resulted in dwarfness of plant height, reduction in internodal distance, spike length and also hastens the maturation period to avoid the effect of adverse environmental variables (Fig.4.12). The number of days taken from emergence to booting, heading and physiological maturity were significantly reduced when sowing was delayed. There was a reduction in the total life span of the crop when sowing was delayed. The relative distribution of the duration of various growth stages and their optimum balance greatly depend on the seasonal conditions under which the crop is grown (**Deng et al.,2000**). An adequately long vegetative stage is required to establish the normal root-shoot system and leaf canopy needed for better crop growth (**Tiwari and Singh, 1995**). In case crop is sown late then flowering/ anthesis period gets shortened considerably because the atmospheric temperature starts rising. Therefore, late sown crop is forced to flower and mature early. The grain growth duration i.e. reproductive phase has negative association with elevated temperature (Fig 4.14), prevailing during this period (**Spano et al .,2003**).

The protein profile indicates that just after anthesis there are several proteins starting from 80kDa to 10kDa in flag leaf of wheat. They start getting degraded / disappearance of many of the proteins as being observed through SDS-PAGE (Fig 4.15). Because, during senescence, many proteases and chlorophyll degradative enzymes function for degrading chlorophyll proteins and other photosynthetic polypeptides. This may be the reason to see less intense protein bands. Since the onset of senescence gets initiated from the upper

part of the leaf and reaches towards the bottom, therefore a leaf was segmented in tip , middle and bottom segments , and used for obtaining its differential SDS-PAGE profile. Interestingly, our data (Fig 4.16) indicates a linear co-relation for down expression of photosynthetic proteins starting from upper leaf segment to lower leaf segment as well as grain protein . Thus, intense and number of protein bands have been observed with bottom and mid segments in contrast to the upper leaf segment. It should also be noted that senescence was induced from the upper segment of leaf. Therefore, protein degradation with the help of internal proteases, clearly favors less intense and certain degraded protein bands of rubisco and D1 protien having molecular weight of 55 and 32kDa (Fig 4.17). These results also indicate that leaf senescence does not occur by massive decay mechanism , but rather is a regulated process that involves co-ordinated expression of specific genes or gene products (**Maria *et al.*, 1997; Buchanan Wollaston, 1997,2003**). Rubisco content increases rapidly during leaf expansion, reaches its maximum around maturation and then declines gradually during senescence (**Michael and Steven, 2004**). It is degraded during senescence and its degradation products are re-utilized as a source of N for developing tissues. The amount of Rubisco in a leaf is the result of a balance between its synthesis and degradation. It has been shown previously that in rice leaves, Rubisco content was very high during leaf expansion, became quite low at the time of full expansion followed by degradation during senescence . The changes in the levels of rbcL and rbcS mRNAs and their relationship to the synthesis of Rubisco has been studied at various points of time starting from expansion to senescence in amaranthus and bean leaves . These observation suggests that the rate of synthesis of large and small subunits of Rubisco is co-regulated both at the transcriptional but also at the translational levels in senescent leaves (**Bota *et al.*, 2004**).

In terms of different yield attributes, high temperature which hastened spike initiation, reduction in internodal length and induction of early flowering in wheat cultivars might have decreased the no. of spikes/m² and grains/ spike mainly by shortening the vegetative growth phase (Fig.4.14), while prevailing high temperature during grain filling might have impaired 1000- grain weight. **Abrol and Ingram (1996)** have also reported that warmer temperature hastens the development, shortens the growth period. Thus, finally lowers the grain yield if the shortened under the prevailing situation of higher temperature. This accelerates respiratory process and inhibits which leads in to poor dry matter accumulation in sink (**Lu et al ., 2001; Singh et al ., 2001; Yang et al., 2004**). The reduction in economic and biological productivity of wheat crop under thermal stress may perhaps be due to reduction in vegetative and reproductive growth phases (Fig.4.18, 4.19). Apart from this, it eventually declined the portioning of biomass from vegetative to reproductive organs as clearly evident from the results of reduced harvest index and grain spike ratio under high temperature (**Yang et al., 2001; Hortensteiner and Feller , 2002**).



Summary and Conclusion.....

Wheat (*Triticum aestivum*, L) is the second most important crop after rice, and is a constituent of major cropping systems across nation. Oftenly, plants are exposed to various kinds of environmental variables which allow either limit or exceed the limit. The adverse environmental variables are basically abiotic stresses viz. light, temperature, humidity, dry air etc. Plant phenotypic acclimation to different combinations of environmental conditions is well known in most crop species (**Le et al ., 2001**). This process of acclimation is very critical for survival and productivity. In our case, wheat as a temperate crop, is chosen to evaluate consequences of elevated temperature and sharp irradiance coupled with dry air causing early maturity followed by forced senescence which consequently impairs crop productivity. Our present study on the effect of high temperature and irradiance causing forced senescence in wheat, provides conclusive evidence of this phenomenon. Efforts were also made to screen out particular genotype/s which could still sustain under adverse environmental variables. To evaluate impact of above mentioned environmental variables, different wheat cultivars were chosen, keeping two sowing schedules i.e. timely (D1) and delayed (D2) sown , with following objectives under consideration:

1. To analyze consequences of high temperature and irradiance on plant performance/ photosynthetic efficiency and plant productivity.
2. Biochemical and ultra structural studies during forced senescence and senescence.
3. Differential behavior of forced senescence affecting plant performance and protein products encoded by photosynthetic genes.

Experiments were conducted with 8 genotypes (Temperature sensitive, UP-2425, UP-2338, PBW-343 and HD-2687; Rainfed, PBW-175 and UP-2113; Hilly genotypes , VL-616 and VL-421) . All

the genotypes were sown, timely (D1) and delayed (D2). After anthesis, different agronomical, morphological, physiological, anatomical, biochemical and molecular parameters were evaluated at different time intervals. The pattern of senescence in relation to different leaf segment of flag leaf and ear head was also investigated for the first time. Finally, qualitative analysis of proteins was carried out with the help of SDS-PAGE, to find out changes in protein pattern associated with date of sowing and senescence, further Western- Blot analysis was done for rubisco large subunit (rbcL) expression.

The major findings are summarized and concluded as follows :

1. The shoot length, spike length, leaf area and internodal distance of all delayed sown cultivars were found to be adversely affected, if sown delayed. Amongst temperature sensitive genotypes UP-2338 has shown maximum shoot and spike length, while this is true for UP-2113 (rainfed) and VL-616 (hilly) genotypes respectively. PBW-175 and VL-421 were under maximally affected genotypes in terms of these two parameters, as they have shown minimum values. The value of flag leaf area was found to be maximum amongst rest of the leaves in all the genotypes. Different internodal distances have shown reduced values under delayed sowing , which varied amongst genotypes. VL-616 has shown maximum value followed by UP-2338.

So, UP-2338 and VL-616 are more or less able to cope under change in environmental variable in terms of growth parameters as mentioned above.

2. Chlorophyll fluorescence variable yield (F_v/F_{max}) which is associated with the functionality of PS II got impaired under delayed sowing conditions. For example if after 20 days of anthesis its value in timely sown crop was 0.638, in delayed sown one it decreased up to 0.214, same was observed in case of ear head (F_v/F_{max}). Besides it, F_v/F_{max} value monitored

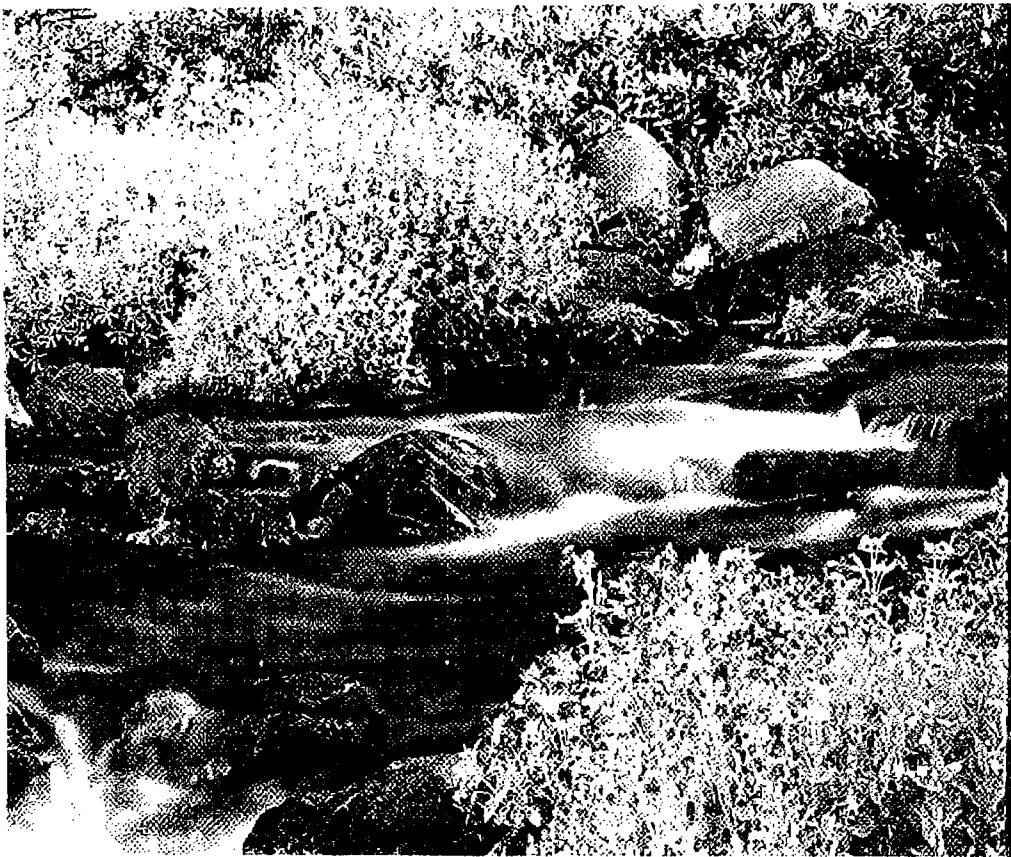
at different segments of flag leaf has shown maximum decline of chlorophyll fluorescence variable yield in tip part, while minimum in the basal part, showing that senescence starts from the upper part of a leaf reaching towards the base and also as the crop attains maturity its PSII efficiency gets decreased which was more prominent in delayed sown cultivars, indicating strong correlation with forced senescence.

Amongst various genotypes, UP-2338, UP-2113 and VL-616 are more efficient in terms of PSII, as compare to PBW-343, PBW-175 and VL-421 under delayed sowing conditions.

3. Photosynthetic pigments viz. chl (a+b) along with chl a/b ratio, have shown reduced value in delayed sown crop as compare to timely sown indicating that forced senescence is responsible for early loss of photosynthetic pigments.
4. Antioxidant enzymes like Superoxide dismutase and Peroxidase activity as well as Malonaldehyde content (for lipid peroxidation) get induced as crop attains maturity. Their expressions were more prominent in delayed sown crop, indicating that abiotic stress (viz. high temperature, sharp irradiance and low humidity/ dry air) has induced the production of antioxidant enzymes which could protect the membrane system due to the production of free radicals. All the genotypes viz. UP-2338, UP-2113 and VL-616 have shown more or less same pattern of their antioxidant enzyme activity as well as MDA content.
5. Ultrastructure of chloroplast at different stages of development has shown various changes. Genotype UP-2338 has been considered at both (D1) and (D2) dates of sowing. Dismantling of chloroplast, unstacking of thylakoid membrane and accumulation of plastoglobules are the symptoms, which arises due to crop maturity / senescence but these are more prominent or earlier in delayed sown condition. This gives strong evidence that under abiotic stress condition or under delayed sowing schedules of high temperature, sharp

irradiance and low humidity, photosynthetic apparatus get adversely affected.

6. The flag leaf is the most potent leaf which is last to come and last to senescence, i.e., it is long lived amongst other leaves. Its rate of senescence was found to be minimum in UP-2338, UP-2113 and VL-616 while PBW-175, PBW-343 and VL-421 have shown early maturity. The overall plant senescence has also followed the same pattern and shown basipetal succession of senescence (i.e., starting from the base). In case of individual leaf, it starts from the tip part. The rate of senescence was found to be accelerated in delayed sown conditions as a result of forced senescence.
7. Due to delayed sowing schedules, plant has completed its life cycle earlier as compare to timely sown one i.e., the days required for both the vegetative and reproductive phases got reduced. Variety, UP-2113 has shown more number of reproductive phase as compare to UP-2338 and VL-616. While VL-616 has shown maximum vegetative days. Both these phases were found to be reduced incase sowing was delayed.
8. Protein profiling of flag leaf and its different segments ie tip ,mid and basal part along with grain protein in both timely and delayed sown crop shows changes in protein patterns as in delayed sown genotypes band pattern was found to be less intense as compared to timely sown crop , specially rbcL (shown by western- blot analysis) and D1 protein.
9. Delayed sowing has adversely affected crop productivity in terms of harvest index (%), grain yield and 1000- grain weight. The maximum value of % harvest index was found in UP-2338 followed by VL-616, while minimum value was monitored in VL-421. The value of grain yield was also found to be maximum in UP-2338 followed by VL-616 and PBW-343. Contrary to this, 1000-grain wt was observed higher in PBW-343 followed by UP-2425. All these yield parameters got reduced if sowing is delayed.



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APPENDICES

APPENDIX-I

(A) Effect of date of sowing on shoot length (cm) in different wheat cultivars

Varieties	D1	D2
HD-2687	80.87	75.08
UP-2113	78.86	71.28
VL-616	82.6	77.26
PBW-343	70.44	68.93
UP-2338	83.5	75.10
PBW-175	77.05	70.42
UP-2425	80.9	77.02
VL-421	65.2	62.8

(B) Effect of date of sowing on spike length (cm) in different wheat cultivars

Varieties	D1	D2
HD-2687	19.62	8.29
UP-2113	12.56	11.63
VL-616	11.78	10.58
PBW-343	9.29	8.09
UP-2338	13.1	12.05
PBW-175	11.26	10.1
UP-2425	11.8	10.54
VL-421	10.8	9.96

(C) Effect of date of sowing on leaf area of leaves at different position, in different wheat cultivars. The values are expressed in cm^2 . The data are means of 5 independent observations. D1 and D2 indicate time and delayed sowing schedules

(a) UP-2338

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	48.0 $\pm$ 3.34	41.0 $\pm$ 3.46
4	37.0 $\pm$ 4.32	34.0 $\pm$ 5.37
3	13.36 $\pm$ 3.71	9.8 $\pm$ 1.70
2	10.38 $\pm$ 1.65	4.8 $\pm$ 1.40
1	3.84 $\pm$ 0.60	3.0 $\pm$ 1.93

(b) PBW-343

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	38.0 $\pm$ 3.46	31.0 $\pm$ 4.40
4	35.0 $\pm$ 5.37	28.0 $\pm$ 3.30
3	11.1 $\pm$ 2.30	8.68 $\pm$ 2.40
2	8.0 $\pm$ 2.90	4.62 $\pm$ 2.83
1	3.65 $\pm$ 1.92	2.6 $\pm$ 1.2

(c) UP-2113

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	52.0 $\pm$ 5.98	48.0 $\pm$ 4.42
4	40.0 $\pm$ 4.46	35.0 $\pm$ 5.37
3	15.86 $\pm$ 2.20	12.03 $\pm$ 5.37
2	11.03 $\pm$ 2.40	5.03 $\pm$ 1.20
1	5.03 $\pm$ 1.40	3.32 $\pm$ 1.20

(d) PBW-175

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	33.0 $\pm$ 3.24	28.0 $\pm$ 4.53
4	27.0 $\pm$ 3.45	22.0 $\pm$ 3.28
3	10.2 $\pm$ 1.50	7.8 $\pm$ 1.60
2	6.50 $\pm$ 1.38	3.15 $\pm$ 1.20
1	3.32 $\pm$ 1.20	2.2 $\pm$ 1.20

(d) VL-616

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	46.0 $\pm$ 5.50	40.0 $\pm$ 4.32
4	35.0 $\pm$ 4.24	30.0 $\pm$ 4.71
3	12.5 $\pm$ 3.10	9.19 $\pm$ 2.32
2	10.1 $\pm$ 2.02	4.08 $\pm$ 2.61
1	4.6 $\pm$ 2.83	3.03 $\pm$ 1.93

(e) VL-421

Leaf position	D1 leaf area cm^2	D2 leaf area cm^2
5	32.00 $\pm$ 4.50	26.00 $\pm$ 3.32
4	25.00 $\pm$ 2.24	16.52 $\pm$ 2.71
3	13.61 $\pm$ 4.10	5.12 $\pm$ 2.30
2	4.66 $\pm$ 2.01	3.0 $\pm$ 2.41
1	2.54 $\pm$ 1.83	2.1 $\pm$ 1.63

(D) Effect of date of sowing on internodal distance different wheat cultivars. The values expressed in cm. . D1 and D2 indicate time and delayed sowing schedules

(a) UP-2338

From top to base	D1	D2
Ist internode	38±1.21	35±0.23
IInd internode	18±0.54	13.6±0.45
IIIrd internode	9.7±0.26	8.5±0.25
IVth internode	6.1±0.45	5.2±0.01

(b) PBW-343

From top to base	D1	D2
Ist internode	30±0.12	28±0.45
IInd internode	10.33±0.22	7.5±0.13
IIIrd internode	8.0±0.15	6.5±0.16
IVth internode	6.6±0.11	3.25±0.21

(c) UP-2113

From top to base	D1	D2
Ist internode	38±0.89	33.5±0.22
IInd internode	17.5±0.16	11.0±0.21
IIIrd internode	12.5±0.36	8.2±0.44
IVth internode	10.0±0.65	6.5±0.22

(d) PBW-175

From top to base	D1	D2
Ist internode	34.5±0.52	28.6±0.15
IInd internode	16.0±0.15	10.5±0.23
IIIrd internode	10.2±0.10	8.5±0.15
IVth internode	6.0±0.05	4.8±0.28

(d) VL-616

From top to base	D1	D2
Ist internode	45±1.2	40.5±0.9
IInd internode	19.5±0.22	18±0.22
IIIrd internode	11.5±0.11	10.2±0.15
IVth internode	6.0±0.25	5.0±0.13

(e) VL-421

From top to base	D1	D2
Ist internode	33.3±0.12	28.2±0.05
IInd internode	15.0±0.05	12.6±0.15
IIIrd internode	9.6±0.03	8.8±0.23
IVth internode	4.8±0.05	4.2±0.45

APPENDIX-II

(A)Effect of date of sowing on Chlorophyll fluorescence variable yield (Fv/ Fmax) of flag leaf in different wheat cultivars. DAA- days after anthesis. D1 and D2 indicate time and delayed sowing schedules

(a) UP-2338

DAA	D1	D2
0	0.848±0.010	0.829±0.012
10	0.829±0.019	0.660±0.018
20	0.638±0.013	0.260±0.014
30	0.427±0.019	0.115±0.003

(b) PBW-343

DAA	D1	D2
0	0.842±0.015	0.750±0.021
10	0.785±0.012	0.320±0.015
20	0.214±0.014	0.101±0.018
30	0.050±0.022	-

(c) UP-2113

DAA	D1	D2
0	0.846±0.010	0.789±0.013
10	0.804±0.019	0.620±0.019
20	0.230±0.013	0.126±0.003
30	0.08±0.009	-

(d) PBW-175

DAA	D1	D2
0	0.836±0.015	0.746±0.012
10	0.736±0.021	0.300±0.022
20	0.104±0.018	0.100±0.014
30	0.050±0.04	-

(d) VL-616

DAA	D1	D2
0	0.832±0.010	0.819±0.015
10	0.813±0.018	0.512±0.012
20	0.320±0.019	0.152±0.015
30	0.083±0.004	-

(e) VL-421

DAA	D1	D2
0	0.800±0.015	0.740±0.018
10	0.700±0.018	0.265±0.022
20	0.185±0.021	0.050±0.02
30	0.053±0.008	-

(D) Effect of sowing schedules (timely and delayed sown) on total chlorophyll content and chlorophyll a/b ratio of flag leaf in different wheat cultivars DAA- days after anthesis. D1 and D2 indicate (timely and delayed sown)

(a) UP-2338

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	4.065±0.23	3.89±0.14	3.460±0.20	3.06±0.06
10	3.751±0.31	3.41±0.09	3.120±0.33	2.83±0.23
20	3.356±0.36	2.88±0.06	2.760±0.20	2.10±0.15
30	2.812±0.26	2.50±0.08	1.51±0.26	1.66±0.20

(a) UP-2113

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	4.065±0.42	3.89±0.16	3.053±0.24	2.83±0.06
10	3.616±0.23	3.51±0.08	2.934±0.30	2.74±0.08
20	3.431±0.30	3.20±0.06	2.532±0.32	2.10±0.32
30	2.72±0.36	2.65±0.05	1.200±0.20	1.50±0.15

(e) VL-616

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	3.712±0.42	3.58±0.13	3.204±0.20	2.81±0.06
10	3.569±0.23	3.20±0.09	2.59±0.33	2.30±0.32
20	3.356±0.31	2.88±0.04	2.50±0.22	2.10±0.15
30	2.48±0.36	2.10±0.05	1.60±0.31	1.14±0.12

(b) PBW-343

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	3.950±0.25	3.76±0.15	3.253±0.28	3.06±0.13
10	3.802±0.33	3.61±0.20	2.753±0.35	2.81±0.22
20	3.225±0.20	3.24±0.14	2.532±0.28	2.00±0.32
30	2.320±0.25	2.00±0.08	1.210±0.31	1.16±0.15

(d) PBW-175

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	3.762±0.25	3.85±0.16	2.956±0.06	3.14±0.16
10	3.563±0.32	3.64±0.12	2.600±0.08	2.83±0.05
20	2.990±0.22	3.45±0.22	2.353±0.32	2.54±0.08
30	2.353±0.32	2.54±0.25	1.413±0.15	1.02±0.15

(f) VL-421

DAA	D1		D2	
	Total chl.	Chl a/b	Total chl.	Chl a/b
0	3.512±0.45	2.88±0.12	3.204±0.22	2.00±0.08
10	2.580±0.26	2.15±0.22	1.85±0.28	1.72±0.25
20	1.80±0.31	1.50±0.28	1.32±0.31	0.86±0.15
30	0.75±0.15	0.82±0.15	0.51±0.22	0.46±0.22

APPENDIX-III

(A) Effect of date of sowing on MDA content in different wheat cultivars. The values are expressed in $\mu\text{M/gm}$ (fr.m.)

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	0.28±0.06	0.35±0.04	0.48±0.004	0.72±0.02	0.35±0.025	0.6±0.05	0.80±.01	1.24±.03
VL-616	0.25±0.05	0.32±0.02	0.42±0.03	0.68±0.012	0.32±0.026	0.46±0.04	0.72±.05	1.25±.028
UP-2113	0.20±0.03	0.26±0.06	0.38±0.04	0.51±0.02	0.25±0.03	0.42±0.01	0.56±.03	0.78±0.03

(B) Effect of date of sowing on Peroxidase activity in different wheat cultivars. The values are expressed in unit / mg of protien

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	4.89±0.5	6.2±0.36	8.2±0.42	11.1±0.15	5.6±0.49	8.5±0.40	12.4±0.3	15.86±0.26
VL-616	4.62±0.41	5.02±0.52	7.12±0.48	10.5±0.41	4.81±0.44	6.12±0.36	8.86±0.3 1	14.22±0.12
UP-2113	5.86±0.4	6.15±0.5	7.89±0.31	10.32±0.26	6.24±0.52	8.62±0.32	10.11±0. 28	12.25±0.31

(C) Effect of date of sowing on Superoxide dismutase activity in different wheat cultivars. The values are expressed in unit/ mg of protien

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	28.6±2.6	30.21±3.15	35±4.23	75±3.6	30±5.2	35.26±6.3	45±5.4	120±4.2
VL-616	22.8±3.5	28.6±4.12	32.8±5.6	62.8±3.25	25.1±2.1	32.65±4.2	43.20±3.62	117.2±4.12
UP-2113	18.6±3.26	23.15±4.41	25.6±3.25	50.12±1.86	20.22±4.2	30.15±2.68	42.26±3.15	100.2±5.1

**(B) Effect of date of sowing on Chlorophyll fluorescence
variable yield (Fv/ Fmax) of different segment of flag leaf
in different wheat cultivars. DAA- days after anthesis. D1 and
D2 indicate time and delayed sowing schedules, T- Tip, M-
Mid, and B- Base**

(a) UP-2338

DAA	D1			D2		
	T	M	B	T	M	B
0	0.850±0.018	0.820±0.016	0.830±0.018	0.536±0.019	0.763±0.010	0.840±0.019
10	0.800±0.020	0.800±0.020	0.810±0.022	0.322±0.011	0.752±0.015	0.844±0.020
20	0.196±0.003	0.750±0.010	0.788±0.015	0.07±0.004	0.400±0.015	0.619±0.019
30	0.02±0.005	0.411±0.015	0.500±0.019	0.0	0.100±0.004	0.300±0.008

(b) PBW- 343

DAA	D1			D2		
	T	M	B	T	M	B
0	0.836±0.020	0.824±0.016	0.838±0.018	0.535±0.019	0.750±0.010	0.838±0.019
10	0.800±0.020	0.795±0.020	0.805±0.022	0.320±0.011	0.742±0.015	0.836±0.020
20	0.184±0.003	0.743±0.010	0.776±0.015	0.07±0.004	0.386±0.015	0.606±0.019
30	0.02±0.005	0.405±0.015	0.486±0.019	0.0	0.100±0.004	0.286±0.008

(c) UP-2113

DAA	D1			D2		
	T	M	B	T	M	B
0	0.850±0.020	0.860±0.016	0.866±0.018	0.500±0.019	0.750±0.010	0.800±0.019
10	0.845±0.020	0.850±0.020	0.861±0.022	0.320±0.011	0.750±0.015	0.800±0.020
20	0.110±0.003	0.805±0.010	0.819±0.015	0.085±0.004	0.300±0.015	0.752±0.019
30	0.08±0.005	0.325±0.015	0.450±0.019	0.60±0.002	0.100±0.004	0.200±0.008

(d) PBW-175

DAA	D1			D2		
	T	M	B	T	M	B
0	0.816±0.020	0.840±0.016	0.852±0.018	0.412±0.019	0.750±0.010	0.800±0.019
10	0.412±0.020	0.798±0.020	0.815±0.022	0.212±0.011	0.617±0.015	0.800±0.020
20	0.111±0.003	0.520±0.010	0.712±0.015	0.07±0.004	0.224±0.015	0.314±0.019
30	0.09±0.005	0.210±0.015	0.286±0.019	0.04±0.002	0.09±0.004	0.119±0.008

(e) VL-616

DAA	D1			D2		
	T	M	B	T	M	B
0	0.810±0.020	0.846±0.016	0.852±0.018	0.337±0.019	0.734±0.010	0.828±0.019
10	0.338±0.020	0.740±0.020	0.829±0.022	0.215±0.011	0.640±0.015	0.780±0.020
20	0.110±0.003	0.113±0.010	0.756±0.015	0.09±0.004	0.470±0.015	0.690±0.019
30	0.02±0.005	0.100±0.015	0.215±0.019	0.04±0.002	0.09±0.004	0.119±0.008

(f) VL-421

DAA	D1			D2		
	T	M	B	T	M	B
0	0.780±0.020	0.815±0.016	0.845±0.018	0.673±0.019	0.794±0.010	0.821±0.019
10	0.210±0.020	0.621±0.020	0.720±0.022	0.01±0.011	0.521±0.015	0.625±0.020
20	0.09±0.003	0.440±0.010	0.512±0.015	0.0	0.315±0.015	0.356±0.019
30	0.0	0.112±0.015	0.198±0.019	0.0	0.09±0.004	0.101±0.008

(C) Effect of date of sowing on Chlorophyll fluorescence variable yield (Fv/ Fmax) of ear head in different wheat cultivars. DAA- days after anthesis. D1 and D2 indicate time and delayed sowing schedules

(a) UP-2338

DAA	D1	D2
0	0.756±0.019	0.728±0.016
10	0.680±0.021	0.609±0.013
20	0.544±0.024	0.434±0.014
30	0.397±0.013	0.219±0.016

(b) PBW-343

DAA	D1	D2
0	0.728±0.045	0.660±0.012
10	0.609±0.015	0.491±0.026
20	0.436±0.032	0.105±0.045
30	0.260±0.012	-

(c) UP-2113

DAA	D1	D2
0	0.765±0.012	0.728±0.045
10	0.686±0.023	0.542±0.021
20	0.548±0.016	0.435±0.025
30	0.420±0.023	0.354±0.012

(d) PBW- 175

DAA	D1	D2
0	0.680±0.056	0.652±0.012
10	0.547±0.023	0.387±0.015
20	0.392±0.028	0.109±0.013
30	0.105±0.018	-

(e) VL-616

DAA	D1	D2
0	0.758±0.025	0.602±0.023
10	0.700±0.023	0.571±0.025
20	0.485±0.015	0.400±0.026
30	0.320±0.025	0.132±0.023

(f) VL-421

DAA	D1	D2
0	0.507±0.023	0.481±0.021
10	0.397±0.013	0.285±0.026
20	0.286±0.026	0.132±0.015
30	-	-

APPENDIX-IV

(A)Effect of date of sowing on % ear head senescence in different wheat cultivars

. DAA- days after anthesis. D1 and D2 indicate time and delayed sowing schedules (at different time intervals)

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	0	15±1.2	35±2.5	75±5.0	0	25±2.5	50±2.0	100
PBW-343	0	25±1.6	40±2.4	85±2.5	10±1.6	40±2.5	65±3.0	100
UP-2113	0	10±1.6	25±2.8	65±5.7	0	25±2.2	40±2.0	100
PBW-175	0	30±2.5	75±5.3	100	10±1.4	50±3.4	100	-
VL-616	0	10±1.2	40±1.5	80±5.0	0	30±2.5	65±3.0	100
VL-421	15±1.5	60±3.0	85±4.6	100	30±2.5	85±4.3	100	-

(B)Effect of date of sowing on % plant senescence in different wheat cultivars

. DAA- days after anthesis. D1 and D2 indicate time and delayed sowing schedules (at different time intervals)

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	7±1.0	21±1.2	35±2.8	77±3.7	25±2.5	45±3.4	75±4.5	100
PBW-343	16±1.2	36±2.6	60±4.0	100	36±2.8	48±3.4	80±3.7	100
UP-2113	8±1.2	20±1.2	37±2.5	76±5.0	22±1.8	39±2.5	46±2.0	100
PBW-175	13±1.5	45±2.8	80±5.0	100	30±2.5	61±4.0	92±1.2	100
VL-616	6±1.0	17±1.6	36±2.5	77±5.0	22±1.4	40±2.6	52±4.5	100
VL-421	11±2.0	37±2.6	78±5.2	100	20±1.5	45±2.8	90±4.5	100

(C) Effect of date of sowing on % flag leaf senescence in different wheat cultivars. DAA- days after anthesis. D1 and D2 indicate time and delayed sowing schedules (at different time intervals)

Varities	D1				D2			
	0	10	20	30	0	10	20	30
UP-2338	0	0	40±2.5	70±4.1	0	10±1.6	60±4.0	90±5.2
PBW-343	0	5±1.0	65±3.0	100	0	15±1.2	80±5.0	100
UP-2113	0	0	35±2.5	60±3.0	0	10±1.8	60±4.0	80±5.0
PBW-175	0	10±1.2	75±5.0	100	0	20±1.6	100	-
VL-616	0	0	40±1.5	80±4.3	0	10±1.5	70±4.1	100
VL-421	0	25±2.5	75±5.0	100	0	60±4.2	100	-

**(D) Effect of date of sowing on Total life span (in days) I- days to anthesis, II- days to maturity , III- total days in different wheat cultivars
D1 and D2 indicate time and delayed sowing schedules**

Varieties	D1			D2		
	I	II	III	I	II	III
UP-2338	93	35	128	87	20	107
PBW-343	95	28	123	89	20	109
UP-2113	93	45	138	87	30	117
PBW-175	85	24	109	79	12	91
VL-616	101	27	128	91	20	111
VL-421	87	25	112	78	22	100

APPENDIX-V

(A) Effect of date of sowing on harvest index (%) in different wheat cultivars. D1 and D2 indicate time and delayed sowing schedules

Varieties	D1	D2
HD-2687	34.13	23.26
UP-2113	34.75	23.63
VL-616	36.62	21.12
PBW-343	36.22	20.40
UP-2338	36.69	23.31
PBW-175	34.27	19.11
UP-2425	36.7	22.20
VL-421	32.51	20.4

(B) Effect of date of sowing on 1000- grain wt (gm) in different wheat cultivars. D1 and D2 indicate time and delayed sowing schedules

Varieties	D1	D2
HD-2687	36.1	31.50
UP-2113	34.9	32.4
VL-616	40.71	37.3
PBW-343	46.46	40.35
UP-2338	37.13	35.0
PBW-175	30.10	25.80
UP-2425	45.00	42.23
VL-421	28.60	23.10

(C) Effect of date of sowing on grain yield (q/ha) in different wheat cultivars. D1 and D2 indicate time and delayed sowing schedules

Varieties	D1	D2
HD-2687	33.27	21.6
UP-2113	35.59	22.9
VL-616	36.8	24.49
PBW-343	36.46	23.15
UP-2338	37.27	24.76
PBW-175	35.5	23.81
UP-2425	35.69	22.90
VL-421	30.12	18.50

(D) Effect of date of sowing on % reduction of different Yield parameters in different wheat cultivars. D1 and D2 indicate time and delayed sowing schedules

Varities	% reduction in 1000-grain wt.	% reduction in harvest index	% reduction in grain yield
HD-2687	12.74	31.8	35.07
UP-2113	7.16	32	35.65
VL-616	8.37	42.32	33.45
PBW-343	13.15	39.53	36.50
UP-2338	5.73	36.46	33.56
PBW-175	14.28	40.47	32.90
UP-2425	6.15	39.5	35.8
VL-421	19.23	41.21	38.57

APPENDIX VI

(A) Analysis of variance on the data on Shoot length (cm) , at the stage of anthesis

Source of Variation	Degree of freedom	Mean sum of squares
		At anthesis (shoot length , cm)
		2003-2004
Replication	2	16.56
Date of sowing	1	454.55**
Error a	2	1.47
Varities	7	91.45**
Interaction	7	19.20**
Error b	28	4.16

*CD at 5%

(B) Analysis of variance on the data on Spike length (cm) , at the stage of anthesis

Source of Variation	Degree of freedom	Mean sum of squares
		At anthesis (spike length , cm)
		2003-2004
Replication	2	0.66
Date of sowing	1	11.95**
Error a	2	0.10
Varities	7	10.26**
Interaction	7	0.31*
Error b	28	0.11

*CD at 5%.

(C) Analysis of variance on the data on leaf area (cm²) , at different positions :

Source of Variation	Degree of freedom	Mean sum of squares				
		Leaf area in cm ²				
		Ist leaf	IIInd leaf	IIIrd leaf	IVth leaf	Flag leaf
Replication	2	0.127	0.64	0.79	2.98	9.65
Date of sowing	1	16.25**	262.74**	125.38**	200.91**	251.39*
Error a	2	4.53	0.43	0.38	1.64	3.39
Varities	7	2.28**	5.54**	13.29**	138.8**	239.75**
Interaction	7	0.35**	1.18**	1.11**	4.28(ns)	3.57(ns)
Error b	28	5.09	0.100	0.107	2.67	3.55

* CD at 5%.

(D) Analysis of variance on the data on different Internodal distance (cm) :

Source of Variation	Degree of freedom	Mean sum of squares		
		Internodal distance (cm)		
		Ist	IIInd	IIIrd
Replication	2	0.75	1.25	0.31
Date of sowing	1	222.01**	46.39**	57.31**
Error a	2	0.242	0.115	0.51
Varities	7	56.14**	7.52**	7.37**
Interaction	7	7.31**	1.26**	2.09**
Error b	28	0.19	0.167	9.5

* CD at 5%.

APPENDIX VII

(A) Analysis of variance on the data on Ear head (Fv/Fmax) at different time intervals :

Source of Variation	Degree of freedom	Mean sum of squares			
		Ear head (Fv/Fmax)			
		0DAA	10DAA	20DAA	30DAA
Replication	2	1557.66	376.06	3671.64	346.19
Date of sowing	1	6.85**	0.255**	0.2837**	0.182**
Error a	2	81583.8	3326.93	1807.01	253.53
Varities	5	3.85**	3.50**	5.82**	0.100**
Interaction	5	65.27**	71.46**	94.52**	1.63**
Error b	20	4801.23	6873.90	1455.6	236.9

* CD at 5%

(B) Analysis of variance on the data on Flag leaf (Fv/Fmax) at different time intervals :

Source of Variation	Degree of freedom	Mean sum of squares			
		Flag leaf (Fv/Fmax)			
		0DAA	10DAA	20DAA	30DAA
Replication	2	7279.71	362.07	72916.35	35688.28
Date of sowing	1	2.51**	0.98**	0.193**	4.96**
Error a	2	5139.22	1810.98	5732.2	1515.63
Varities	5	34.64**	7.17**	9.65**	6.45**
Interaction	5	17.62**	2.65**	2.33**	2.23**
Error b	20	1224.9	2624.31	3944.6	79579.6

* CD at 5%

APPENDIX VIII

(A) Analysis of variance on the data on % Flag leaf senescence at different time intervals :

Source of Variation	Degree of freedom	Mean sum of squares	
		Flag leaf % senescence	
		20 DAA	30 DAA
Replication	2	72.76	0.109
Date of sowing	1	1788.51(ns)	86979.8**
Error a	2	106.02	0.4765
Varities	7	928.61*	167.32**
Interaction	7	188.76(ns)	752.91**
Error b	28	290.06	2.02

* CD at 5%.

(B) Analysis of variance on the data on % plant senescence at different time intervals :

Source of Variation	Degree of freedom	Mean sum of squares			
		% Plant senescence			
		0DAA	10DAA	20DAA	30DAA
Replication	2	1.89	6.41	35.78	21.64
Date of sowing	1	151.51**	192.58*	1921.55*	3536.32**
Error a	2	0.201	9.61	31.14	21.65
Varieties	7	464.28**	1473.9**	1103.46**	117.47**
Interaction	7	157.98**	203.04**	268.51**	117.47**
Error b	28	1.98	2.50	3.21	1.43

* CD at 5%.

(C) Analysis of variance on the data on % Ear head senescence at different time intervals :

Source of Variation	Degree of freedom	Mean sum of squares		
		% ear head senescence		
		10 DAA	20 DAA	30 DAA
Replication	2	19.75	20.81	29.64
Date of sowing	1	3456.10**	5418.75**	14595.19**
Error a	2	1.58	6.25	34.18
Varieties	7	556.09**	2357.77**	1064.79**
Interaction	7	20.68**	42.54**	135.07(ns)
Error b	28	4.23	8.32	120.42

* CD at 5%.

APPENDIX IX

(A) Analysis of variance on the data on life span (days) :

Source of Variation	Degree of freedom	Mean sum of squares		
		Life span (days)		
		Vegetative	Reproductive	Total
Replication	2	49.18	0.645	47.78
Date of sowing	1	481.33**	728.52**	2227.70**
Error a	2	2.77	0.2711	13.17
Varities	7	374.76**	172.61**	821.76**
Interaction	7	9.00*	33.09**	26.16*
Error b	28	3.19	2.148	8.97

*
CD at 5%

APPENDIX X

(A) Analysis of variance on the data on % Harvest index :

Source of Variation	Degree of freedom	Mean sum of squares
		Harvest index (%)
		2003-2004
Replication	2	4.87
Date of sowing	1	2160.20**
Error a	2	6.84
Varities	7	2.75(ns)
Interaction	7	4.95(ns)
Error b	28	2.82

* CD at 5%.

(B) Analysis of variance on the data on Grain yield (q/ha):

Source of Variation	Degree of freedom	Mean sum of squares
		Grain yield (q/ha)
		2003-2004
Replication	2	2.28
Date of sowing	1	1813.62**
Error a	2	0.973
Varities	7	6.37**
Interaction	7	1.00
Error b	28	1.89

* CD at 5%.

(C) Analysis of variance on the data on 1000-grain wt (gm) :

Source of Variation	Degree of freedom	Mean sum of squares
		1000-grain wt (gm)
		2003-2004
Replication	2	6.44
Date of sowing	1	187.23**
Error a	2	1.30
Varities	7	181.69**
Interaction	7	3.30(ns)
Error b	28	1.57

* CD at 5%.

Appendix XI

Environmental variables during the crop season November 2003 to April 2004

Meteorological wk.		Mean temperature (0 C)		Rainfall (mm)	Relative humidity (%)		Sunshine (hrs.)
Wk no.	Wk.dt	Max.	Min.		Max.(7AM)	Min. (2PM)	
44 Nov	1-7	22.17	11.96	0	91.86	46.5	7.58
45Nov	8-14	26.28	9.8	0.03	88.43	38	18.68
46Nov	15-21	25.5	7.04	0	92.14	38.4	8.4
47Dec	22-28	23.9	7.3	0	94	94.7	7.4
48Dec	29-5	24.18	10.58	0	88	50.4	4.7
49Dec	6-12	21.6	10.4	2.05	91.14	62.4	3.6
50Dec	13-19	16.8	7.2	0.6	92.8	64.1	3.7
51Dec	20-26	14.4	7.9	0	93.3	7.3	3.6
52Jan	27-2	14.6	7.5	0	92.5	76	1.57
53Jan	3-9	18.28	5.76	0	97	61.4	5.24
54 Jan	10-16	18.7	8.94	0	79.4	65.6	3.67
55 Jan	17-23	18.3	17.8	2.34	96.2	67.8	4.11
56 Jan	24-30	18.8	7.9	0	93.6	60.6	4.4
57 Feb	1-7	17.27	6.27	0.486	95.7	62.29	3.8
58 Feb	8-12	23.56	8.7	0	92.29	49.29	8.3
59 Feb	15-21	25.12	9.37	0	86.71	48	8.2
60 Feb	22-28	26.1	10.61	0	90.86	50.86	8.36
61March	1-7	27.16	8.66	0	91.14	38.14	10.05
62March	8-14	29.5	13.44	0	83.71	37	9.3
63March	15-21	32.66	16.28	0	87.14	44.7	8.4
64March	22-28	35.18	15.46	0	78.86	21.71	9.5
65April	29-5	35.9	17.08	0	69.42	25.71	8.8
66April	6-12	37.98	19.07	0	62.42	21.57	9.32
67April	13-19	38.15	19.12	0	59.57	19.57	6.9
68April	20-26	32.98	19.21	2.06	66.14	39.14	6.12

VITA

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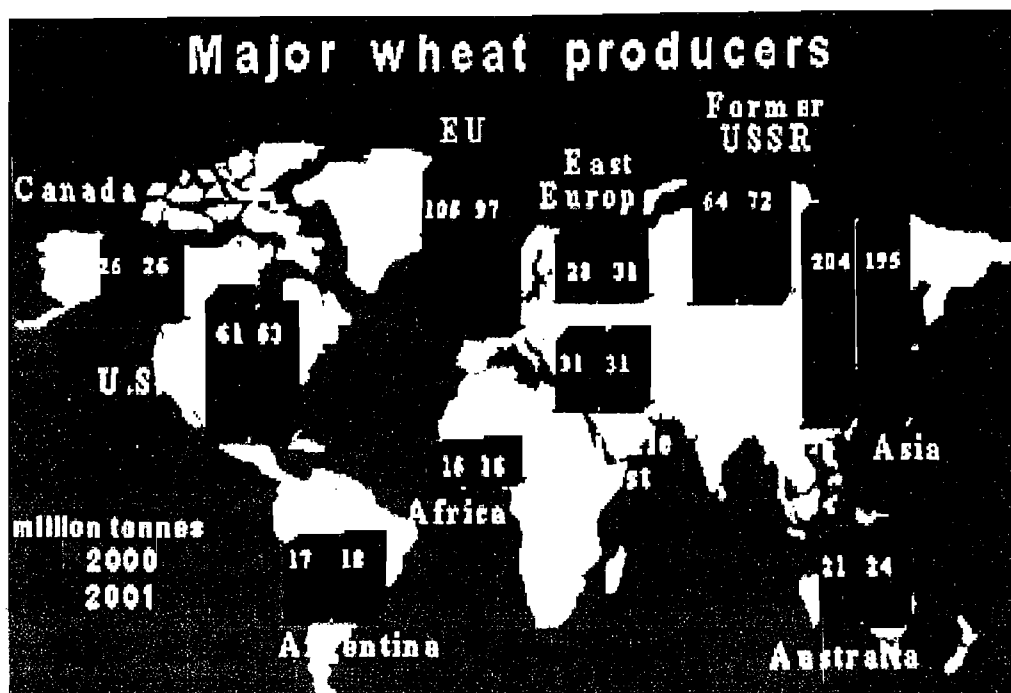
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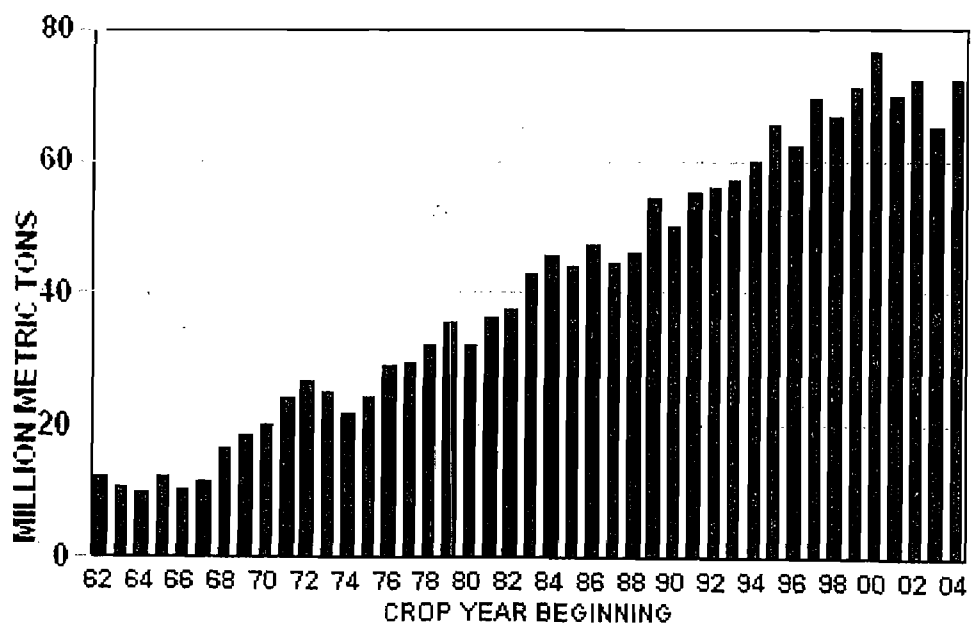
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INDIA WHEAT PRODUCTION



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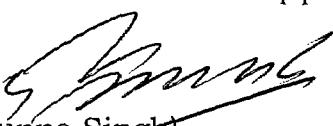
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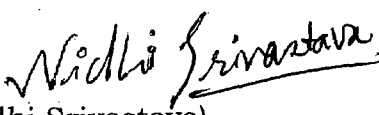
Abstract

Wheat is a major cereal crop of North India. The phenophases of wheat get developed under temperate climatic conditions. However, in case plant experiences rise in temperature during anthesis coupled with sharp irradiance, high temperature and low humidity, may express decline in photosynthetic CO_2 assimilation and chlorophyll fluorescence variable yield (F_v/F_{max}). In view of this field experiments were conducted using eight wheat genotypes (UP-2338, PBW-343, HD-2687, UP-2113, PBW-175, UP-2425, VL-616 and VL-421), keeping the sowing gap of 20 days. The delayed sowing negatively contributed for plant development viz. plant height, spike length, leaf area and internodal distance. The value of photosynthetic pigments viz. chl (a+b) along with chl a/b ratio also got reduced if sowing schedule was delayed. Chlorophyll fluorescence variable Yield (F_v/f_{max}), which indicates the functionality of PSII also got impaired as plant attains maturity in flag leaf, ear head and in different segments of flag leaf. The value of F_v/F_{max} started to reduce from the upper part of the plant or a leaf, showing the basipetal succession of senescence in wheat.

Different antioxidant enzymes viz Superoxide dismutase and peroxidase got produced at the maturation of the crop, but their induction was earlier under delayed sown crop. Peroxidation of membrane lipids get accelerated under high temperature stress, parallel to our result of delayed sown crop. The chloroplast ultrastructure has also shown different peculiar features, during senescence viz. unstacking of thylakoid membrane, accumulation of plastoglobules etc. These abnormalities were more prominent or earlier under delayed sowing condition. The SDS- PAGE protein profile pattern of flag leaf at different time intervals indicates about the prominent appearance of a band having almost 55K Da molecular size, specifically expressed for Rubisco LSU. Apart from this, several bands seen during anthesis which get either disappeared or diffused, associated with the maturity schedule. Further, Western- Blot analysis of Rubisco favors that LSU encoded by rbc L of plastid genome gets expressed maximally during anthesis. However, its expression gets down-regulated with maturity/ senescence.

Finally the yield attributes in terms of harvest index, grain yield and 1000- grain weight indicate that the delayed sowing unfavorably contributes for these parameters. The forced senescence also gets switched-on in all delayed sown wheat genotypes, which enhances early maturity with impaired plant performance. The detail findings presented here would be useful to design crop modelling to overcome from the negative impact of elevated temperature associated with loss in crop productivity.


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