Morpho-physiological and biochemical characterization of wheat varieties (*Triticum aestivum* L.) sown at differential time intervals

Thesis

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By

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Tanvi chandra

Pantnagar
September, 2021

(Tanvi Chandra)
Authoress

CERTIFICATE

This is to certify that the thesis entitled **“Morpho-physiological and biochemical characterization of wheat varieties (*Triticum aestivum* L.) sown at differential time intervals”** 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 & Technology, Pantnagar, is a record of bonafide research carried out by **Ms. Tanvi Chandra, Id. No. 49564,** 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 have been duly acknowledged.

Pantnagar,
September, 2021

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We, the undersigned, members of the Advisory Committee of **Ms. Tanvi Chandra, Id. No. 49564** 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 "**Morpho-physiological and biochemical characterization of wheat varieties (*Triticum aestivum* L.) sown at differential time intervals**" may be submitted in partial fulfillment of the requirements for the degree.

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LIST OF SYMBOLS

ml	:	Milli litre
cm	:	Centimeter
Kg	:	Kilo grams
ppm	:	parts per million
%	:	Percentage
°C	:	Degree Celcius
L	:	Litre
g	:	Gram
µg	:	Microgram
mg	:	Milli gram
nm	:	nano meter
M	:	Moles
KDa	:	Kilo Dalton
m^2	:	Square metre
Kg	:	Kilogram
t/ha	:	tons per hectare
Tg	:	Tera gram
MT	:	Metric Tons
FW	:	Fresh weight
DW	:	Dry weight
LAI	:	Leaf area index
TDM	:	Total dry matter
CF	:	Chlorophyll Fluorescence
CTD	:	Canopy Temperature Depression
15 DAA	:	15 Days after ANTHEISS
RWC	:	Relative Water Content
MSI	:	Membrane Stability Index
SOD	:	Super oxide dis mutase
CAT	:	Catalase
HSP	:	Heat shock proteins
D1	:	Sowing in month of November
D2	:	Sowing in month of December

Introduction

Chapter 1 INTRODUCTION

Wheat (*Triticum aestivum* L. member of family *Poaceae*) is a major cereal crop in many parts of the world and occupies a major place in the cereal economy of the world and particularly India as well. North India cultivates wheat as a major cereal crop during winters. About 13.64% of wheat production in world wheat is produced by India. Major wheat growing zones of India are Northern Hill Zone (NHZ), North Western Plains Zone (NWPZ), North Eastern Plains Zone (NEPZ), Central Zone, and Peninsular Zone **(Ramadas *et al.*, 2020).** This crop performs well under temperature climatic variables with low rain fall (**Kimber *et al.*, 1987**). Nutritionally, wheat includes 12.1% protein, 1.8% ash, 2.0% reducing sugars, 6.7% pentosans, 59.2% starch, 70% total carbohydrates. It is also a good source of minerals and vitamins viz., calcium (37mg/100mg), iron (4.1mg/100mg), thiamine (0.45mg/100mg), riboflavin (0.13mg/100mg) and nicotinic acid (5.4mg/100mg) (**Lorenz *et al.*, 1991**). In economical point of view, raw wheat used as flour, hard durum wheat as semolina, germinated and dried wheat as malt, crushed and cracked wheat as parboiled (or steamed), dried, crushed and de-branned wheat as bulgur (groats), even outer husk/bran also used in a several ways. Quality characteristics which make wheat cultivation more useful are milling yield, rheological properties bread making properties (**Souza *et al.*, 2004**). Global wheat production is approximately 774.30 million metric ton and the forecast demand to fulfill the need of growing world population varies between 840-1050 million ton. According to FAO 2018, nearly 749.5Tg of wheat were produced in 2017 on nearly 200 million ha with grain yield level of 3.4 t/ha. In 2050, the world's population is expected to reach 9 billion and demand of wheat will increase to >900 Tg (**Tadess, *et al.*, 2019; Statistica, 2021).**

In the last few decades climate change is changing our habitat in which our agricultural crops and different agronomic practices are changing rapidly. Being a winter crop, variability in climates is one of the biggest environmental threats to Indian wheat basically in area like Gangetic plains, central and peninsular parts. As per the projection earth's atmospheric temperature is increased by 2 ºC every year.

Research also predicted that with every 1°C rise in temperature, the wheat production will be decreased by 4–6 million tonnes. Rainfed wheat will also experience a reduction in yield with 9–25% profit loss for every 2–3.5°C elevation in temperature (**Statistica, 2021)**. In addition to the production of wheat is also affected by other constraints such as escalating population, leads to increasing demand of food, competition for cultivable land, proper irrigation, intercropping cropping system, excessive use of chemicals, pest and disease complex, less availability of improved seed (**Sharma *et al*., 2014).** According to various experimental studies, the impact of climate change in wheat production was analyzed and concluded that elevated temperatures negatively affect the growth and developmental process of wheat plant thus resulting in severely hampered productivity. Fluctuation in temperature extremes and water deficient conditions other abiotic factors, strong light, elevated temperatures, global warming makes earth unsuitable for yield production and fulfilling the calorie demands of growing population (**Iqbal *et al*., 2017).**

Sowing timing, is another most influential constraints that limit wheat productivity directly as delay in sowing conditions ultimately results in exposure of plants to higher temperatures during anthesis and grain filling stage of wheat life cycle. Under intercropping system such as rice-wheat, late onset of monsoon due to climatic variations leads to delay in rice sowing. As a result of it, wheat sowing also delayed which adversely affects its yield. Rise in temperature along with dry hot air affects performance of ear head and flag leaf of winter wheat in relation to assimilation and translocation of photosynthates into developing grains. Heat stress also results in forced senescence of foliage and poor assimilates synthesis (**Yang *et al*., 2004**). Elevation in temperature disturbs the metabolic processes and responsible for affecting various physiochemical morphological and biochemical traits (**Farrant *et al*., 2004).** A considerable decrease in economic yield was observed when wheat crop expose to high temperature condition during its floral initiation and spikelet development stage thus adversely affecting the maximum yield potential of the crop. Sink strength and source capacity were found vital factors in modifying yield quantity and quality during heat shock (**Dia *et al*., 2016**). Elevation in temperatures also affect various phenological stages and process such as germination, emergence, leaf stem development and growth, tiller number, dry matter production, floral initiation, panicle exertion, pollination, fertilization, seed growth, seed yield and seed quality of winter wheat. Higher temperature just before anthesis showed significant increase in floral abortion, pollen sterility, loss of seed set, rupturing of pollen tube, and loss of pollen viability. Both micro and mega-sporogenesis process were found affected. Heat stress during post anthesis results reduced grain number, grain weight and altered grain quality by affecting availability and translocation of photosynthates to developing kernel, reduced starch synthesis and deposition within kernel **(Iqbal *et al*., 2017**).

Capability of plants to produce good yield under elevated temperature is known as heat tolerance. Plants are sessile, so they have natural ability to adapt towards stressful condition or develop/increase some thermo-tolerant traits their survival. There are various actions/mechanisms by which plant avoid/adapt harsh environment. Heat induced damage leads leaf rolling, leaf shedding, reducing leaf size. Leaf thickening, reducing growth duration, transpiration cooling and other adjustments related to morphology and ontogeny (**Wahid *et al*., 2007).** Development of antioxidant system (enzymatic and non-enzymatic), stay green traits (maintenance relative water content, canopy temperature, membrane stability, chlorophyll fluorescence, chlorophyll pigments and carotenoids, for longer duration in plant life cycle) are some of the features of plants that show thermo-tolerance (**Harris *et al*., 2008**).The molecular target of heat stress involves protein denaturation, damage to lipid (increment in malondialdehyde content), nucleic acid and other molecules due to production of reactive oxygen species. Plants overcome by synthesizing various protective molecules such as stress chaperon, storage proteins (albumin, globulin, gladian and glutens), total protein (in grains) and developing the heat tolerant characteristics in them (**Qu *et al*., 2013**). Least reduction in enzymes such as nitrate reductase activity and increased osmotic adjustments (proline content) in leaves also helps in finding biochemical aspects of wheat cultivars under stressed and non-stressed conditions. To improve the crop production in terms of calorie demands and differential environmental conditions, efforts had been made towards finding the appropriate sowing timing of varieties, tolerant cultivars with high heat flexibility characteristics, varieties which can also give acceptable yield results under both sowing conditions, varieties showed least reduction in yield attributes and in which seed nutritional quality least hampered due to elevation in temperatures (**Hatfield and Prueger, 2015**). Findings regarding the morphological, physiological and biochemical aspects for heat sensitivity and tolerant behavior of crops can be used for assessment in the development of new selection criteria for breading programs. As these traits are stable, easy to measure, heritable and strongly correlated with the yield (**Priya *et al*., 2018).**

Keeping all the facts in the mind present study was conducted with the following objectives:

OBJECTIVES;

1) To evaluate the morphological and physiological parameters of differentially sown wheat varieties.
2) To study the yield and yield attributes of differentially sown wheat varieties.
3) To evaluate the biochemical parameters and enzymatic analysis of wheat varieties under differentially sown conditions.
4) To evaluate the effect of heat stress on quality and seed storage proteins.

Review of Literature

Chapter 2 REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L.), a cereal grain (caryopsis) considered as a staple food for almost half of the world's population. Many species of wheat together make up one genus called ***Triticum*** however common wheat (*T. aestivum*) is grown widely across the world. According to archaeological records, wheat was first cultivated in Levant range (region of the Fertile Crescent; south-eastern turkey) around 9600 BCE **(Belderok *et al.*, 2000**). The earliest cultivated forms were diploid (genome AA) (Einkorn wheat) and tetraploid (genome AABB) (Emmer wheat) (**Tanno *et al.*, 2006**). With evolution, a repeated round of hybridization took place between cultivated Emmer wheat and an unrelated wild grass *Triticum tauschii* (also called *Aegilops tauschii* and *Ae. squarosa*) that led to developing a novel hexaploidy wheat (genome AABBDD) which was selected by farmers for its superior properties such as larger grains, seeds (inside the spikelet) that remain attached to an ear by toughened rachis during harvesting while in wild wheat, more fragile rachis were present that allow the ear to easily shatter and disperse the spiklets (**Dubcovsky and Dvorak, 2007**). Normally about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% are tetraploid durum wheat (**Shewry, 2009**).

In 1960's when industrialization and westernization of diet was introduced in the world, suddenly global production and demand of wheat increases because it has unique viscoelastic and adhesive properties of gluten protein that facilitates the production of processed food such as bread, muffins, noodles, pasta, biscuits, cookies, cakes etc. in market and for fermentation to make beer and other beverages or biofuel. As a result, it become the second most important cereal grain after maize worldwide (**Day *et al.*, 2006**). Globally, wheat production is about 772.64 million metric tons in 2020-2021 and increased over 30 million tons as compared to previous market years. China is the major producer of wheat with a record production of 5.48 tons/ha followed by India (3.37 tons/ha), Russia (3.11 tons/ha) and USA (3.12 tons/ha). Other traditional wheat growing countries are Canada, Ukraine Pakistan, New Zealand, Zambia and Mexico **(Ramadas *et al.*, 2020; Statistica, 2021**).

India is blessed with diverse agroecological conditions. In India, wheat cultivation was introduced 5000 years ago and gradually becomes top 10 wheat producing countries of the world, in terms of area it is in 1st position, but after independence, India has faced a net deficit in food production and had to import wheat for domestic consumption from other countries. During 1966-67, India adopts a new strategy called Green revolution for improving the production of wheat and rice and as a result of several developmental and food security-based programs now it became the second largest producer of wheat worldwide, next to China (**Sharma *et al.*, 2014**). From past few years the production of wheat has increased significantly from 75.81 million MT in 2006-7, 87.39 million MT in 2012-13 to 94.88 million MT in 2017-18 **(Ramadas *et al.*, 2020)**. Data depicts that the total area under wheat crop was about 29.80 million hectares in 2018/19 and 29.31 million hectares in 2019/20. As now, value reaches to 31.35 million hectares in 2020/21. Major wheat producing states are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Bihar and Rajasthan **(U.S.D.A, 2021).**

Climatic requirements

Wheat is winter season rabi crop. It is grown in tropical, sub-tropical as well as temperate zones and cold tracts of country. It can tolerate severe cold and snow and resumes its growth when conditions become favorable and can be cultivated from sea level to as high as 3300 meters. Undoubtedly, this wide range adaptation has been due to complex nature of its genome which provide great plasticity to the crop **(Samra and Singh, 2004).** The best weather conditions for wheat cultivation are in areas that have cool, moist weather during the major portion of the growing period followed by dry, warm weather to enable the grain to ripen properly. The optimum temperature range ideal for wheat growth and development is 20 to 25 ºC. Temperature below 3.5 ºC during germination and above 35 ºC during grain filling & maturation will results lower germination rate and reduced grain yield respectively. Wheat can be grown in regions where rainfall varies from 25 to 150 cm per year with a require of medium (50-60%) humidity for their growth but during the time of maturity crop requires less humidity land warm season. Soils with a clay loam or loam texture, good structure and moderate water holding capacity are ideal for wheat cultivation. The time and placement of fertilizer is another area where significant progress has been made to increase crop productivity. It was demonstrated that 150 kg nitrogen, 60 kg phosphorus and 40 kg potash per hectare were required for optimum productivity of wheat (**Dhyani, 2010**).

Factors affecting the crop growth and development

The various growth stages during wheat life cycle are usually distinguished as; germination, emergence, tillering, floral initiation or double ridge, terminal spikelet, first internode or beginning of stem elongation, booting, spike emergence, anthesis and maturity and broadly classify as; germination to emergence (E), emergence to double ridge (GROWTH STAGE 1), double ridge to anthesis (GROWTH STAGE 2) and (GROWTH STAGE 3) from anthesis to maturity which includes grain filling period (**Hanft and Wych, 1982**). These growth stages are highly affected by various factors such as biotic, abiotic stresses, application of herbicides, pesticides, irrigation etc. (**Oerke, 1999**). Abiotic stresses mainly strong light, too low or elevated temperature, solar radiation conditions soil salinity, waterlogging, inadequate mineral nutrition, toxic heavy metal, oxidative stress and drought may adversely affect plant growth and development by affecting all essential cellular activities such as loss of photosynthesis activity, macromolecule hydrolyzation, oxidative injury, nutrient deficiencyin wheat plant (**Thakur *et al*., 2016**).

Among abiotic stresses, extreme temperatures are known to be the most important environmental variable that drives the various metabolic processes during wheat development **(Ortiz *et al*., 2008**). Evidences showed that the minimum air temperature across the globe becomes above normal while global mean surface temperature increased by 0.5 degree Celsius in 20th century and it is projected to increase by 1.8 to 4.5 degree Celsius by 2100 (**Janjua *et al*., 2010).** While according to latest finding via global climate model, the mean ambient temperature is likely to rise by 6^{o}C by the end of 21st century. According to various reports, it was observed that the variations in average growing season temperatures of ± 2°C during the main wheat growing regions could cause reductions in grain production of up to 50%. It is because of increased leaf senescence as a result of elevated temperatures >34°C (**Poudel and Poudel, 2020**).

For wheat, the optimum temperatures for various developmental stages are as follows; emergence to tillering (16 to 22.6 °C), tillering stage to heading (15.4-21.8°C) and heading to maturity (17.5-20.4°C) any fluctuations from these optimum temperatures are considered as heatstress or heat shock to the plant that can cause irreversible damage and ultimately affects yield potential of the crop (**Akter and Islam, 2017**). Studies showed that elevated temperature causes decrease in net photosynthetic amount by reducing photosynthetic pigments, stomatal conductance proportion, enzyme activity and acceleration senescence and shortening growth cycle of crop (**Hasan *et al*., 2019**).

Apart from abiotic stresses, climate change is another an uncontrollable factor which have severe impact on wheat growth and deeply influences wheat productivity. The major climatic variables are cloud cover, diurnal temperature range, precipitation, temperature and vapour pressure (**Dalmia, 2004)**. Change in climate is due to various natural and anthropogenic activities such as deforestation, use of fossil fuel, increasing CO_2 concentration (from 280 ppm, to 380 ppm), industrialization, causes increase in greenhouse gases and global warming which results temperature extremes and water deficient conditions to the crop for longer period and cause irreversible damage & considered as heat stress to the plant hence hampered growth, development and yield (**Mitchell and Jones, 2005**). Due to climate change, frequency and magnitude of extreme weather events increases rapidly and responsible for damaging yield potential of crop (**Solomon *et al*., 2007; Iqbal *et al*., 2017**).

An appropriate sowing timing is also another most important factor for ensuring optimum productivity in wheat cultivar **(Sandhu *et al*., 1999).** Generally, vegetative phase in wheat life cycle is normally under control of low temperature and less humidity but as reproductive phase of wheat begins the temperature starts rising and as a result of delay in sowing conditions plant experience the exposure of high temperature (heat stress) during its anthesis and grain- filling stage. This causes induction of forced senescence of foliage, poor assimilate synthesis, reduced translocation of photosynthates to the developing grains and great respiratory loss, ultimately affects the productivity and quality of the crop **(Kanchan and Mahendra, 2000).** A delay by 30 days in sowing timing could significantly reduce the period from sowing to anthesis and anthesis to maturity duration. Early sown or appropriate sown wheat varieties had high number of days for anthesis and maturity than late sown **(Virender & Sharma, 2000).** The greater number of days for heading and maturity were taken by the crop which sown on early or in appropriate dates is favors for better translocation for photosynthates from source to sink. Whereas, crops under late sowing experience shortened flowering period and grain filling period as the atmospheric temperature starts rising sharply, consequently in late sown crop forced to flower and mature early **(Sikder and Hossain, 2001).** In India, under rice-wheat intercropping system, late onset of monsoon due to climate change leads to delay in rice sowing. As a result of it, wheat sowing also delayed which adversely affects its yield (**Iijima*et al* 2005)**. According to previous reports, it was analyzed that cultivars sown at appropriate timings had longer post heading period and therefore longer grain filling period than late sowing cultivars. Additionally, early sowing cultivars faces grain filling period during lower air temperature. Another advantage of earlier sowing cultivar is that they produce fewer tillers and a smaller number of leaves but retained more green leaves for longer time than late sown cultivars (**Iqbal *et al*., 2017**). Late sown crop cultivars have a later filling stage compared with early sown crop cultivars hence, late sowing has been adopted as a method to induce heat stress in field condition. According to an experiment done in contrasting lentil genotypes i.e. two heat-tolerant (HT; 1G 2507 and 1G 4258) and two heat-sensitive (HS; 1G 3973 and 1G 3964) under two sowing conditions i.e. (2nd week of November 2014) and (2nd week of February 2015) and as a result of delay in sowing an increment of temperature >30º/20C (day/night) was found imposed on the genotypes during their pollination and seed filling stage and which considered as heat stress to them. Through research it was found that the maximum and minimum temperatures during seed filling for the normal sowing treatment were found below 30/20°C [22–30°C (day)/16–19°C (night)] while due to delayed sowing treatment the temperature were above 30/20°C [32–40°C (day)/21–27°C (night)] which results decrement in Leaf and seed sucrose, seed number and seed weight per plant in heat sensitive genotype as compared to heat tolerant (**Sehgal *et al*., 2017**). But in some part of the country such as eastern Indo-Gangetic plains in India late sowing of wheat is a common practice due to late harvesting of long-duration rice varieties hence there is need to know the appropriate timing of sowing of various wheat varieties so to suggest the breeders that which would perform better with early sowing, timely sowing or late sowing conditions (**Dwivedi *et al*., 2018).**

2.1 Effect of elevated temperatures on sensitive stages of wheat life cycle

Inappropriate sowing timings or delay in sowing can cause severe cellular injury, catastrophic collapse of cellular organization, hinderance in proper functioning of cell or even cell death (**Schoffle *et al*., 1999**). In most wheat growing areas, the crop experiences the highest temperatures during anthesis and grain filling hence these two stages are considered as the most sensitive in wheat life cycle. Optimum temperature for wheat during anthesis according to various researches is 23ºC and minimum to maximum temperature range during anthesis is 9.3 to 32 ºC. Temperature above the mentioned range considered as heat stress. An experiment was conducted to study the effect of high-temperatures when applied during the anthesis stage in wheat (*Triticum aestivum* (L.) cv. Chablis. The results showed 40% reduction in biomass accumulation, 41% in tiller number, 45% gain weight, 40% in harvest index, 25% in nitrogen content (in leaves) due to elevated temperature at the time of anthesis (**Wollenweber *et al*., 2003)**. Heat stress during anthesis stage also leads to disintegration of photosystem-II, chlorophyll pigment, rupturing of pollens, dryness in stigmatic surface, flower abortion, increased photorespiration, lower CO_2 assimilation, denaturation of key pathways associated with various enzyme activity, empty pockets in endospermic tissue, defragmented starch granules, improper transportation of photosynthates and ultimately shriveled seed grains (**Kumar *et al*., 2013**; **Feller and Vaseva, 2014**). Studies indicated that average maximum temperature above higher than 32ºC during reproductive period of wheat development could cause yield loss of 30% and sometime cause complete sterility of florets (**Zhongfu *et al*., 2018**). During an experiment abnormal anthers development (both structurally and functionally in 80% florets) was reported when wheat plant was exposed to 30ºC for 3-day period around anthesis, (**Dwivedi *et al*., 2018).** In an experiment, two spring wheat lineswere sown under earlier plantings and late planting. Data revealed that under early planting, plants emerged slowly, which increased the total length of time to reach anthesis and to reach maturity along with cool soil temperature during anthesis stage results no heat stress during that period and plant took longer to emerge. The earliest planting results 124.70 days from planting to reach maturity while the delayed planting only required 104.70 days. The difference of 20 days results 14 more days to emerge than late plantation and 6.6 days longer to progress from emergence to maturity. The longer duration from emergence to anthesis and from anthesis to maturity for the earlier planted treatments included vegetative growth periods up to 3 days longer, and grain filling periods up 4 days longer than the late planted treatments. **(Collier *et al.*, 2021).**

Elevated temperatures due to delay in sowing during grain filling causes denaturation of enzymes associated with source and sink. The optimum temperature for filling grains for wheat is 21.3ºC while minimum to maximum temperature range for grain filling is 9.36 to 34.3 ºC. In two different wheat cultivars, Yangmai 9 and Xuzhou 26 when exposed to different temperatures (34°C/ 22°C, 32°C/24°C, 26°C/14°C, and 24°C/16°C) during grain filling the enzymes related to plant defense system (SOD and CAT) were found decreased under high-temperature treatment (34°C/22°C) compared with optimum temperature treatment. Reduction in soluble proteins and chlorophyll content in flag leaves were also recorded. Grain-filling rate initially increased with elevation in temperatures but decreased significantly under higher temperatures (**Zhao *et al.*, 2007).** Heat stress accelerates the rate of grain filling whereas, filling duration becomes shorter, for instance, six wheat (*Triticum aestivum* L.) genotypes were investigated in climate-controlled greenhouses under two different temperatures (day/night: 20°C/15°C and 25°C/20°C). A rise of 5 °C in temperature reduces the duration of grain filling by 12 days (>30%). Supply of photo assimilates at this time also found limited (**Yin *et al.*, 2009**). Three major events in wheat grain development i.e. assimilate translocation, filling duration and rate is depend on ambient temperature. During grain filling assimilates transferred to grains by two ways; either by current assimilation or by pre anthesis stored stem reserves. About 90% of carbon transferred to grains via current assimilation. Under heat stress, photosynthetic source of assimilation of assimilates reduced and alternative source (stem reserves remobilization) increases from 6 to 100%. Current photosynthesis provides 63 to 65% of assimilates to grain at 20/15ºC and 30/25ºC day/night temperature respectively (**Farooq *et al.*, 2011).** According to a research, late sowing cause elevated temperatures during post anthesis stage that significantly reduced the duration of grain filling by 1-2 weeks showing heat reduces the duration of filling wheat grain (**Farooq *et al.*, 2011).** Yield loss due to post flowering heat stress is mainly due to abortion of grains and decreased grain weight (**Lobell *et al.*, 2012**). Reduced grain weight and grain quality in wheat is also due to elevated temperatures during grain filling as it affects availability and translocation of photosynthates to developing grains, starch synthesis and deposition within grains (**Iqbal *et al.*, 2017**). Elevated temperatures also cause grain shrinking in wheat through ultra- structural changes in aleurone layer and endosperm cells it occurs when the day and night temperature increased from 25/14 ºC to 31/20 ºC under post anthesis stage (**Zhongfu *et al.*, 2018)**. In another research, an experiment was conducted in 237 spring wheat under two growing seasons 2015-16 and 2016-17 with October sown and February sown conditions. February sown conditions results in filling stage post-ponded by 2 -3 weeks and resulted in increase of 2ºC in average temperature during grain filling stage. In addition, late sowing (February) significantly reduces the duration of grain filling by 1-2 weeks. (**Wang *et al.*, 2018).** In an experiment, Chinese spring wheat is subjected to heat stress, and results showed that grains under heat stress reaches to maturity by 30 day from flowering while under control condition grain reaches to maturity by 38 days after flowering. A shortage of 8 days was observed (**Wang *et al.*, 2018).** Identification and development of wheat varieties that can grow under heat stress conditions is a need of hour. According to an experiment, 58 wheat genotypes were planted at optimal and late sowing condition. Results revealed that wheat sown at optimal condition had better growth and yield-related traits, and lead to better grain yield. While under late sown (heat stress conditions), grain yield of all tested genotypes was significantly decreased. Genotypes ‘Norin’ and ‘Eagle rock’ were found susceptible to high temperature, while genotypes ‘Misr-1’ and ‘Misr-2’ were tolerant and produced highest yield under late sown conditions. While ‘Misr-2’ found performed better under both sowing condition which shows the adaptive behaviour of Misr-2’ towards elevated temperature. According to this research, genotypes ‘Misr-2’, ‘Misr-1’ and ‘Shandaweel-1’ may be recommended for planting under hot environmental conditions tolerant varieties **(Youldash *et al*., 2020).**

2.2 Effect of elevated temperatures on morphological parameters

The morphological parameters are highly influenced by elevated temperatures and found responsible for causing big differences in yielding capacity of different wheat varieties (**Singh *et al*., 1984).** In previous researches, plant height, internodal distance, tiller number, productive tillers are considered as good indicators of overall health and yield potential of the crops because if plant height is appropriate more photosynthates are stored in stem and translocated to grains during grain filling stage while more tiller numbers lead to possibility of more productive tillers and good yield at maturity. In contrast, the reason for low production and productivity in wheat is tall plants as sometimes due to tall plants resulting in lodging, poor tiller and low sink capacity, high susceptible to diseases, highly sensitive to thermo and photo variations, poor adaptability, longer crop duration results long exposure of plant to climate variations and insect/pest/disease attacks (**Stones and Nicolas, 1994**). According to previous research in wheat, cv. HD2285, P10066, P10423 and WH291 that were sown on three different sowing timings (25 Dec., 4 Jan. and 15 Jan), it was found that under delayed sowing, reduction in plant stand and number of tillers was observed during maximum tillering stage. Number of productive tillers also noticed to be reduced under delayed sowing conditions (**Bisnoi and Taneja, 1990**). As delayed sowing causes heat stress during growth period that affects wheat morphology by declining shoot dry mass, relative growth rate, and leaf expansion of different wheat cultivar (**Nelson, 1998**). Other researches also concludes that high temperature prevailing during anthesis leads to force maturity, reduces leaf area, plant height, internodal distance and tiller number when wheat plant grown lately (**Kanchan and Mahendra, 2000**). In contrast, according to one research, 8 different wheat cultivars were grown under ambient and hyper thermal stress (+ 3.0 °C) conditions to study the effect of elevated temperatures on growth and yield response. Under high temperature stress, an increment of 10.71% in average leaf area and 14.28 % in average internodal distance while a reduction of 28.12 % in leaf area index and 6.33 % in leaf width was observed **(Singh *et al*., 2001).** Maintenance of internodal distance is also associated with plant height, previous research shows that reduction in plant height due to heat stress is mainly due to reduced differential loss of internodal length of various crop species. First internodal distance seems to be more affected by delayed sowing however in contrast, increase in internodal distance also observed in some crop species under delayed sowing condition (**Singh and Singh, 2000**). An experiment was done in 10 spring wheat genotypes to study the effect of elevated temperatures on their morphological traits. The results showed that due to late sowing (elevated temperatures) on an average, the yield in all genotypes were reduced upto 53.75%, tiller number also found reduced upto 15.38% (**Riaz-ud-Din *et al.*, 2010)**. According to another research, reduced number of tillers with promoted shoot elongation was observed in different wheat varieties when they are under stress condition as compared to normal conditions (**Farooq *et al.*, 2011**). In an experiment, a reduction of 13.85% in PAK-81, 8.70% in LU-26S, 3.70% in Sunalika wheat varieties was observed in plant height due to high temperature stress during pre-anthesis stage (**Ali *et al.*, 2016**). The impact of high temperature on growth and productivity in crop is observed by analyzing 18 genotypes under different sowing regimes with an interval of 30 days. The results showed that high temperature stress due to late sowing has negative influence on plant growth and yield due to the modulation of various physiological and biochemical activities, a reduction of 8% in plant height was observed under late sowing as compared to optimal condition. Among the genotypes, NIA-AS-14-9, NIA-Sunder, Raskoh, TJ-83, Dani, Saher-2006 and SH-Salt-5B produced high plant biomass, productive tillers, number of grains and grain yield per plant under both sowing conditions and found suitable for timely as well as late sowing. However, SH-Salt-6, NRL-1236, NIA-Sarang, LU-26s and Dani were found failed to cope up elevated temperature and showed poor performance under late sowing (**Khan *et al.*, 2020).**

The importance of flag leaf in controlling plant growth and productivity is already explained by previous scientists who concluded that the rate of leaf area expansion has greater influence on dry matter accumulation in various crop species. flag leaf as powerful source of assimilates, help in translocation of photosynthates to developing grains. However, it has been found to be most affected by heat stress, results reduced assimilate supplies to ear head (**Viana and Mitiver, 1980**). An experiment was conducted in nine different crop species (maize, cotton, soyabean, velvet leaf, spurred anoda, prickly sida, cocklerbur, johnsongrass and pigweed) which were sown under three temperature regimes with day/night temperatures of 21°C/10°C, 32°C /21°C, and 38°C /27 °C. Under elevated temperatures (38°C /27), relative growth rate, relative leaf area expansion rates, relative leaf weight growth rates were significantly and negatively affected in comparison to temperature regime (32°C /21°C) (**Potter and Jones, 1997).** In an experiment, higher leaf area index and leaf area of different wheat varieties was observed under normal sowing (November 22) as compared to elevated temperatures (December 22) condition. A reduction of 23.03% and 23.27 % in leaf area index while 44.96 % and 45.81 % in leaf area was observed under elevated conditions during two consecutive years 2000-2001 and 2001-2002 during anthesis. Highest LAI was noticed in PBW343 under normal sowing and in UP2425 under elevated temperatures (**Mishra, 2002).** Such reduction is may be due to some anatomical changes took place in plants under higher temperature such as reduction in cell size, partial closure of stomata for restriction towards water loss, increased number of xylem vessels in roots and shoots that lead to have negative effect on growth and development of plant. The harmful effect of heat stress on leaf can also be observed in terms of visible scorching of leaves and twings, leaf senescence, lesser canopy growth along with poor yield (**Vollenweider, 2005**). At sub cellular level, alteration in chloroplast by negatively regulated stacking of grana and structural organization of thylakoids cause variation in photosynthetic process leads to reduction of various morphological parameters in wheat (**Sing, 2009).** In an experiment different wheat cultivar were grown under elevated temperature of 35 °C showed significant increase in average leaf area, leaf area per shoot, leaf weight ratio and leaf length whereas, it reduces the specific leaf weight, leaf width, total dry weight **(Dwivedi *et al*., 2018).** In a recent study, different wheat genotypes were analyzed based on their correlation between their yield related traits and grain yield and it was found that the traits related to flag leaf were found non-significantly affected by elevated temperatures while flag leaf area (cm^2) of different wheat varieties had strong correlation with their grain yield shows elevated temperature may or may not negatively affects agro-traits related to flag leaf. A reduction of 21.60% in flag leaf area and 7.30% in flag leaf wide was observed under stress condition as compared to optimal condition (**Youldash e*t al*., 2020).**

2.3 Effect of elevated temperatures on yield and yield attributes

Thermo-tolerant nature of yield attributes of any variety/genotype makes it suitable to sown in elevated temperatures, achieved maximum output and found satisfied by yield potential (**Evan, 1998**). Previous findings shows that elevated temperatures due to inappropriate sowing timings decreases the yield parameters and having negative correlation with them which ultimately decreases the yield of crop. As due to delay in sowing, heat stress affects the days to appearance of first node in crop species, reduce tiller number per plant, thousand grain weight and grain yield per plant. So sowing condition is found an important aspect which regulates the plant performance and productivity (**Singha *et al*., 2006**)**.** In an experiment, spike length and spike weight found negatively affected by sowing timings as normally sown (20 November) wheat genotypes had longer spike length in comparison to late sown (23 December) wheat genotypes. A reduction of 2.11% and 39.13% in spike length was observed under late plantation as compared to normal while a reduction of 2.48% to 32.59% in spike weight was also observed under late sowing condition as compared to normal sowing **(Dhyani, 2010).** An experiment was conducted with 6 different wheat genotypes under two conditions; plants-maintained heat stress (inside the glass house at 35-40°C for 3 hour) and normal (kept in the open atmosphere). The results showed 2.17% to 7.92% reduction in number of spikelets per spike 22.02% to 27.10% in number of grains per spike and 5.87 % to 19.86 % in yield under elevated temperature as compared to normal conditions (**Ali *et al*., 2016**). Similarly, a reduction of 8.4% in biomass, 33.90% in grain yield, 28% in harvest index, 4.30% in grain weight, 30.90% in grains/m^2, 25.30% in grains/spike, 13.10 in spikelet per spike, 8% in spike/m^2 and 13.50% in peduncle length was observed under late planting (March 1, 2016) as compared to optimal planting condition (October 1, 2015). While in spike length 4.2% increment due to late sowing was observed. The correlational analysis was also done between various yield-related traits of 18 different wheat genotypes which concludes that the most of the traits had significant influence on grain yield under both sowing conditions. Biomass and grain yield components were found positively and more significantly related but, spike number/area was negatively and significantly related to grain yield. The relation between peduncle length, kernel weight and kernel number were also found significant and strong with each other. Spikelet number/spike and spike length were found more significant under heat stress than non-stressed condition. Grain number/spike and grain weight, had strong positive relationship with the grain yield under non-stressed condition while under stressed condition, grain number (per spike), grain weight and flag leaf area had strong correlation with grain yield (**Youldash *et al*., 2020).**

Delay in sowing directly regulates seed biomass, spike length and spike weight. As grain number and grain weight are highly sensitive towards heat stress, temperature above 20ºC between spike initiation and anthesis reduce grain number per spike (**Ferris *et al*., 1998**). Grain number also influenced by phenostage of plant development which include spikelet initiation, floral organ differentiation, male female sporogenesis, pollination and fertilization. The reason is that heat stress speed up the development of spike causes reduced spikelet number and thus number of fertile spikelets (grains per spike) (**Saini and Aspinall, 1982, Porter and Gawith, 1999**). However, the period between spike initiation and anthesis is not too sensitive to elevated temperatures but the period between the appearance of double ridges on shoot apex and flag leaf is found too sensitive. This sensitivity is due to the reduction of duration of emergence to double ridges and double ridges to anthesis lead to reduction in spikelet number per spike and grain number per spikelet. An experiment was conducted in which 14 spring wheat genotypes were exposed to heat stress from 30 to 38ºC causes reduction of grain weight by 20 to 44% (**Tahir and Nakata, 2005**). High temperature >30ºC during floret development and floret initiation can cause reduction in grain number or sometimes complete sterility in some wheat cultivars (**Anjum *et al*., 2008**). Elevated temperature also causes shrinking of grains through changes in ultra-structure inside aleurone layer and endosperm cells of kernel when day night temperature increased from 25/14ºC to 31/20ºC **(Dias *et al*., 2008)**. Every single degree elevation in temperature lead to a decrease of 4% of grain number per spike. Inadequate supply of a assimilates could also be a reason for floret sterility that led to decrement in grain number per spike. If wheat plant experience high temperature >30C during pollen mother cells are dividing substantially, reduce grain set and therefore grain yield (**Farooq *et al*., 2011**). Heat stress also impairs viability of anther and pollen, resulting in poor fertilization. The sensitivity of pollen towards high temperature is due to they are found incapable to produce HSPs in response to heat stress. Elevated temperature during meiosis and growth of ovaries could also be as reason for reduction in grain weight in wheat crop under delayed sowing (**Farooq *et al*., 2011**). Heat stress also found responsible for disturbance in kernel morphology by compacting the duration of grain filling period. According to previous report on the post-anthesis heat stress on head trait of wheat showed that various parameters such as grain filling duration, head weight, kernel weight were reduced in comparison to control but kernel number remains unaffected (**Iqbal *et al*., 2017**).An experiment was done to analyze the effect of terminal heat stress on various yield attributes of different wheat varieties under two crop seasons planting dates, normal (mid-November) and late sown (late December) with proper irrigated environments. The results shows that due to late sowing (heat stress) grain yield were the most affected trait among others (>30% reduction), whereas traits like filling rate, grain number/spike and thousand kernel weight (11.40%) were less affected (<15%). Based on the performance of genotypes under both sowing conditions WH 1021, NW 1014 and NW 2036 were found as the heat tolerant wheat genotypes, while HD3086, HD 2967 and HD 3059 were found suitable for both normal and heat stress environments. The reason for the difference in performance of various genotypes under both sowing conditions is maybe due to 8.1% reduction in days to anthesis and 13% in days to maturity which cause shortening of growth phase and filling duration that ultimately led to forced senescence of crop (**Kumar *et al*., 2020**). In a recent investigation, two spring wheat cultivars (Millet-11, Punjab-11) &(V-07096, V-10110) were investigated in response to terminal heat stress (due to delayed sowing condition).Based on their morpho-physiological traits, the results concludes that delayed sowing causes reduction in spike length (13%), number of grains per spike (10%), 1000-grain weight (13%) and biological yield (15–20%) as compared to timely sowing condition in all wheat cultivars. However, higher productive tillers (19–20%) and grain yield (9%) were recorded under late sowing. According to the research, Punjab-11 found as better agronomic performance as compared to other cultivars as it has delayed maturity with proper grain filling period **(Tariq *et al.*, 2021).**

Heat susceptibility Index (HSI) helps in determining the stress tolerance in terms of minimizing the decrease in production caused by unfavorable and favorable conditions (**Asana and Williams, 1965).** From the ratio of plant performance under heat stress and optimum temperatures heat tolerance behavior of that particular variety/genotype can be easily identified. According to one research, it is reported that genetic resistance to heat is apparent when a genotype is more productive than another genotype in environment where stress occurs (**Fisher and Maurer 1978**). On comparing between two genotypes, a sensitive one is significantly different to tolerant one during their booting and during grain filling stage in wheat. The genotypes having high positive value of HSI are susceptible to higher temperatures and vice versa. In peninsular India, high temperature strongly influences wheat growth and yield because under late plantation stress intensity was server and there is reduction in duration of GS2 and GS3 phases (**Hanchinal *et al.*, 1994**). Kernel weight and Kernel size reduction are the best measurement of heat tolerance in wheat, so with the help of heat susceptibility index for grain yield per genotype is an effective measure to evaluate the thermo-tolerance characteristic of various genotypes (**Mohammadi 2004**). According to previous researches, HSI also been used as an indicator of yield stability and a proxy for heat tolerance. In an experiment, four wheat cultivars (Aghrani, Kanchan, CB-30 and Sonora) were evaluated under two sowing conditions i.e. November 30th and December 30th as normal and post anthesis heat stress condition. Heat susceptibility index based on grain yield were found diverse for different wheat cultivars. Results showed that cultivar CB-30 with H.S.I (0.346) was found to be heat tolerant, while cultivar Aghrani (0.962) and Kanchan (0.90) were moderately tolerant and Sonora with H.S.I (1.721) was considered as susceptible towards elevated temperatures (**Hassan *et al.*, 2007**). In another experiment,8 different spring wheat cultivars were investigated for their thermotolerant nature under 3 sowing conditions (early, late and very late). Heat susceptibility index was calculated for grain yield in each case. The results concludes that wheat cultivar (Sufi with H.S.I = 0.35) was highly heat stress-tolerant followed by (BARI Gom-26 with

H.S.I = 0.65) and (Shatabdi H.S.I = 0.62). Other cultivars with H.S.I = >1 was found susceptible towards heat stress (**Hossain *et al*., 2013).** In another crop specie such as cotton, temperature above 36°C during reproductive stage badly affects plant growth and development. 58 different cotton genotypes were studied for tolerance behavior, which were sown at the month of march (experiencing heat stress during their reproductive stage, above 44°C). They were screened on the basis of heat susceptibility index. The values of heat susceptibility index in present research ranged from (0.32 to 10.85). Out of 58 genotypes, 17 were considered as heat tolerant as they showed the values of H.S.I <1.Cotton genotype ‘Sadori’ was found highly heat tolerant with least H.S.I (0.32) (**Abro *et al*., 2015**). An experiment was done in wheat under normal sown & late sown conditions in which genotypes Raj 3765, WH 1080 and WH 1142 showed minimum HSI for a number of traits and almost all genotype of triticale group have minimum values of HSI representing high temperature tolerance of these genotypes (**Suresh *et al*., 2018**). In a recent study it was concluded that the genotype having H.S.I (<0.5) are highly stress tolerant, those having H.S.I (0.5> HSI < l.0) are moderately tolerant, and those having H.S.I (> 1.0) are intolerant. The genotypic response with respect to H.S.I varied from 0.05 to 1.34. Wheat genotypes; Altınözü (1.28), Axe (1.29), Eagle Rock (1.35), Osmaniyem (1.24) and Sagittatio (1.32) were found sensitive to higher temperatures with higher values of H.S.I. while, genotypes Alibey (0.68), Genç99 (0.56) and Kayra (0.70) were found resistant towards heat stress (having lower H.S.I). Similarly, genotypes Shirogane Kamugi (0.05) and Chukogu-146 (0.058) were more resistant (having lowest value of H.S.I) to heat stress due to early maturity (**Youldash *et al*., 2020)**.

2.4. Effect of elevated temperatures on physiological parameters

Physiological parameters such as relative water content, membrane stability index, canopy temperature depression, and chlorophyll ‘a’ fluorescence variable yield of flag leaf is considered as a good marker for identifying thermotolerant behavior of various crop species. According to previous studies in wheat they are found to be highly influenced by increasing temperature due to delayed sowing conditions (**Stones and Nicolas, 1994**). With the help of such parameters the extent of plant performance under elevated temperatures can be easily analyzed.

2.4.1 Relative water content (RWC)

Plant water status is the most important variable under changing ambient temperatures. Under field conditions, high temperature stress is frequently associated with reduced water availability. During dry environment, higher temperature causes high vapor pressure deficit, which drive higher evapotranspiration. If soil water decreases due to elevated temperature, leaf relative water content and leaf water potential also found decreased (**Machado and Paulsen, 2001**). RWC estimates the current water content of sample leaf tissue relative to the maximum water content it can hold at fully turgidity and due to heat stress (delayed sowing), various results indicates that the RWC found to be decreased in leaves of various crop species (**Mazorra *et al*., 2002**) because plants tend to maintain stable tissue water status regardless of temperature when moisture is ample; however, high temperature severely impairs this tendency when water is limiting. Enhanced transpiration during day time induces water deficiency causing a decrease in water potential and leading to perturbation of many physiological processes that affects the productivity of crop (**Tsukaguchi *et al*., 2003**). Plants have tendency to conserve water and make balance between heat balance, stomatal regulation and limited soil water when ambient temperature exceeds 30 ºC **(Martinez-Ballesta, 2009)**. But there is limited information about wheat dynamics for reproductive and grain filling stage in relation to water content and heat balance. However, through previous reports it was observed that the leaf relative water content and leaf water potential were not affected by elevated temperatures when soil water content is close to field capacity. As soil water content decreases by 18% (due to elevated temperatures and uncontrolled soil evaporation) leaf relative water content and leaf water potential also decreases and ultimately leaf senescence occurs and plant died. A negative relation between transpiration rate and increasing temperature (from 15/10ºC to 40/30 ºC) was also found with decreased growth in various crop species. According to an experiment, higher temperature (35/25ºC) after tillering stage in two wheat genotypes leads to a significant reduction in water potential at anthesis, 7 and 15 days after anthesis which concludes that the investigated varieties were susceptible to heats stress (**Almenselman *et al*., 2009).** During anthesis water is required for stem and peduncle elongation, for raising ear up, cell expansion, growth of all parts of ear initiation of flowering (pollen ripening, rapid extension of stamen filament and fertilization while during grain filling water is essential for grain growth and grain filling. Elevated temperature increases hydraulic conductivity of membrane which increases aquaporine activity, membrane fluidity, permeability and reduces water viscosity which is found beneficial for roots and leaves of plants help in minimizing changes in leaf water potential and opened stomata for longer time but alternatively, excess increase in permeability of membrane due to heat stress causes dehydration of flower and grains **(Farooq *et al*., 2011).** According to an experiment, the RWC values were found higher under normal sowing condition and tested above than > 95% in all wheat genotypes while under delayed sowing, the values of RWC were found reduced and ranged between 80 to 93%. Maximum RWC observed in Salt-6 (99.36%) and LU-26s (98.81%) under normal sowing while genotype TJ-83 had maximum RWC under late sowing (92.83%) followed by RN-09111 (92.39%) and KIRAN-95, NRL-1236 (92.28%). Minimum RWC values under late sowing were recorded for genotype NIA-AS-14-10 (82.8%). The results conclude that normal sown plants have higher water contents as compared to stressed-plants, hence, RWC may provide a direct measure of cellular water contents **(Mahboob *et al*., 2015).** The results also revealed that wheat genotypes TJ-83, SRN-09111, KIRAN-95 and NRL-1236 were more effective to maintain high water contents & this could be because of better water conductance of the plasma membrane which consequently ameliorates water uptake (**Khan *et al*., 2020).**

2.4.2 Membrane stability index (MSI)

MSI is a good parameter for screening wheat varieties for heat tolerance. As it is considered as rapid screening method (**Blum, 1988**). Heat induce damage to plasma membrane can easily assayed by MSI which measures electrolytic leakage from leaf tissues after exposure to high temperature. According to some experiments it was proved that membrane thermo stability test could be a suitable measure for selection of heat tolerant wheat genotypes as it is an indicator of heat tolerant behavior of plants (**Saadallla, *et al*., 1993**). Six different wheat genotypes ‘TAM 107’, ‘TAM 108’, ‘Arlin’, ‘Kauz’, ‘Glennson 82’, and ‘Siete Cerros’were tested against two temperature conditions i.e 17 °C and 49°C for 30 min (in water bath) to check their membrane stability index.Their correlational studies concludes that the membrane stability and grain yield have positive correlation with each other (**Ibrahim and Quick, 2001).** An experiment was performed with nine wheat varieties, high MSI was recorded in HD2733, K9006, HP1761, NW1067 and NW1021 under heat stress whereas, in Halna, K8962, NW1076 and NW1041 showed less MSI in vegetative as well as in reproductive stage. Halna was found to be highly tolerant toward heat stress and showed lesser MSI as compared to others (**Singh *et al*., 2007).** To identify the tolerant one among five different wheat genotypes membrane thermostability test based on percent leakage of electrolytes in flag leaves was used under heat stress condition. Among the crosses, PBW452×LoK 54 was found the most tolerant one by least relative injury of 26% as compared to either parents. The study was based on the fact that under high temperature, leakage of ions occurred due to disturbance in cell membrane which is identified by measurement of electrical conductivity measures (**Reddy *et al*., 2008**). The membrane stability index was found lower in heat stress treated PBW343 and C306 genotypes of wheat during anthesis and 15 days after anthesis and a greater reduction was observed in PBW343 (heat sensitive) than in C306 (heat tolerant) (**Almeselmani, 2009**).To investigate the effect of heat stress on MSI of flag leaf in different wheat cultivar, an experiment was conducted with different wheat genotypes; Halna, PBW 343, NW 1014, DBW 16, K 911 and AAI 11 under different sowing conditions; late sowing (25 January) and normal sowing (23 November 2015). The results revealed that the wheat genotypes Halna, NW 1014, DBW 16, K 911, and AAI 11 had high membrane stability index (MSI) with less percent reduction in yield and yield components comparatively to PBW 343 and DBW 16. So, these genotypes with higher MSI can be used as physiologically screened genotypes for heat tolerance because tolerant plant showed less leakage due to accumulation of high saturated fatty acids and monounsaturated fatty acids. (**Jaiswal *et al*., 2017**). Elevated temperature due to delayed sowing causes approximately 2 to 3ºC elevation in temperature as compare to normal sowing, resulting a significant decrease in cell membrane thermo stability index. During experiment different genotypes of wheat were tested under optimal sowing condition and delayed sowing condition. The results revealed that under normal sowing MSI of different wheat genotypes were ranged from 29 to 70.6%. While under delayed sowing, MSI was found decreased and become only 9.1%. Maximum MSI values under optimal sowing condition were recorded in genotype SRN-09111 (70.6%) and Dani (63.7%). But both genotypes could not maintain the higher values of their MSI under heat stress condition and a showed 87.11% relative decrease. Observations regarding the experiment concludes that the MSI clearly showed a significant reduction on the exposure to high temperature due to delayed sowing. The reason for such drastic reduction in values of MSI under heat stress as due to increase in temperature beyond the threshold level could cause an increase in kinetic energy of molecules across membranes resulting loosening in cell membranes either because of increasing unsaturated fatty acids or due to denaturation of proteins **(Khan *et al.*, 2020).**

2.4.3 Canopy temperature depression (CTD)

Canopy temperature depression CTD is an indirect measure of yield potential of crop plants which represents metabolic fitness under specific environmental conditions. It is a remote sensing crop water status using infrared radiation thermometer **(Reynold *et al.*, 2001**). It is rapid, non-destructive and considered as good indicator for water level and strongly correlated with the solar radiations. Even single reading can articulate the temperature scores of leaves, thus decreasing experimental error **(Balota *et al.*, 2008**) so CTD value is also used to selection criteria for tolerance towards high temperature in various crop species especially in wheat (**Kumar *et al.*, 2012**). According to an experiment, four heat tolerant (Gouran, Sourav, Kanchan and Shatabdi) and two heat sensitive (Sonora and Kalyansona) wheat genotypes were tested under normal as well as late sown condition, and it was found that heat tolerant lines exhibited higher CTD than heat sensitive lines suggested that heat tolerant cultivars retained cooler canopies than heat-sensitive cultivars (**Sikder and Paul, 2010**). According to a study, canopy temperature is a single lost cost measurement for selecting heat tolerance in various crop genotypes. In a field experiment at northwest Mexico, 18 breeding trials total 504 spring wheat lines were studied under both heat and drought stressed conditions. Under the heat treatment, canopy temperature was significantly correlated with yield ($r = -0.26$) for all trials. This result indicates that canopy temperature has ability of to predict the yield potential of crops. As cooler canopy temperatures found responsible for enhanced yield potential in wheat crop when exposed to warmer temperature (**Mason *et al*., 2014).** Similar results also occurred in 40 wheat varieties that were tested for heat sensitivity in two growing seasons of (2009–10 and 2010–11), with two sowing dates i.e early (last week of October) and late (first week of December). They also suggested that high CTD value during vegetative stage is an important selection parameter, which gives the idea of reduced evapo-transpiration under early sown cultivars and responsible for increased grain yield whereas, high CTD value at flowering stage contributes to maintain the stay green trait for longer period and responsible for reduced the loss due to evapo-transpiration and in return increased grain yield under late sown conditions (**Saxena *et al*., 2014**). It is also reported that lower canopy temperature during grain filling period is an important physiological principle for high temperature stress tolerance in various crop species such as cotton, wheat etc. (**Mason and Singh 2014**). All tolerant lines had high CTD scores during vegetative stage, which reduces transpiration loss and helped to retain the stay green trait for longer further enhancing yield potential (**Saxena *et al*., 2014).** CTD is highly useful trait for genetic improvement and necessary for breeders to enhance accuracy and speed of genetic gain. From previous reports a positive correlation also been reported between the CTD values and heat resilience and it is also revealed that in wheat, CTD along with flag leaf photosynthesis and stomatal conductance together positively corelated with grain yield under stress condition (**Priya *et al*., 2018**). An experiment was conducted with 18 different wheat genotypes to evaluate the effect different sowing condition on Canopy Temperature Depression (CTD) which was determined at 18 days after anthesis under normal, moderate high and high temperature growing condition. The results concludes that 18 days after anthesis all the wheat genotypes maintains cooler canopy under normal growing condition as compared to both moderate and high temperature sowing conditions. On comparing within genotypes, under high temperature growing condition, BARI Gom-26 and BAW-1202 maintain cooler canopy comparatively than BAW-1182 and BARI Gom-27 and considered as temperature tolerant genotype. The reason for increase in CTD values due to increase respiration and transpiration resulting from stomatal closure. The superior performance of higher canopy depression under heat stress could be due to increased stay green duration with high chlorophyll content that enhance photosynthetic activity in tolerant genotypes (**Sharmin *et al*., 2020**).

2.4.4 Chlorophyll a fluorescence variable yield (Fv/Fmax)

Chlorophyll fluorescence (Fv/Fmax) is an effective and non-damaging tool for elucidating various aspects of physiological state of photosynthetic apparatus (includes Photosystem II and Photosystem I mediated electron transfer and calvin cycle activity) in intact leaves of higher plant (**Thomas and Smart, 1993)**. It was also analyzed that the “Stay green phenotypes” retained photosynthetic competence for longer time than heat treated (delayed sown) or controlled condition plants (normal sown)**.** It helps in estimating the rate of photosynthetic electron transfer and photosynthetic quantum yield as well as capacity of thermal energy dissipation under high heat stress (**Spano *et al*., 2003).** It is the ratio of initial/ground state fluorescence (Fv) to maximum fluorescence (Fmax) of plant leaf. For a healthy plant the Fv/Fmax ratio is estimated as 0.8. Lower level of Fv/Fmax reflects abiotic/biotic stress in plants (**Sayed, 2003)**. According to an experiment in different wheat varieties, a reduction of 26% in total yield was observed due to heat stress as compared to plants under controlled growth chamber. The reason for such declination was found due to the reduction in photosynthetic efficiency which was analyzed by observing the declination in chlorophyll fluorescence values under both stressed and non-stressed conditions (**Hassan, 2006**). The inhibition of PSII electron transport indicated by sharp increase in basal level of chlorophyll fluorescence that led to photosynthetic inhibition. Heat stress also damages PSII active site, separate LHCII from PSII core complex (**Ristic *et al*., 2007**). By some experiment it was observed that Fv/Fmax ratio of flag leaves in wheat decreased after 14 days of anthesis (**Cai *et al*., 2008**). As photosynthesis depends on the function of light harvesting and electron transport system within chloroplast which is expressed as photochemical efficiency measured by chlorophyll a fluorescence variable yield (Fv/Fmax) ratio. This can be variably correlated with photochemical yield of PSII. Previous reports observed that the loss of photosynthetic competence can be a result of several processes such as breakdown of proteins (PSII and Rubisco) and by destruction of membranes by lipid degradation **(Prasad *et al*., 2008ab)**. Effect of heat stress at 37 °C and 45 ºC for 8 hours on seedling of Karacadag and Firat wheat cultivars was observed and it was found that the chlorophyll florescence was significantly decreased in heat treatment (45 ºC) (**Efeoglu *et al.,* 2009).** Previous reports suggest that damage to PSII leads to reduced carbon fixation, oxygen evolution and disturbs linear flow of electrons in thylakoid membrane. The most important reason of PSII sensitivity to high temperature is under heat stress thylakoid membrane fluidity and electron transport dependent integrity of PSII (**Farooq *et al*., 2011).** In a field experiment, 30 wheat genotypes were analyzed for PSII thermostability by using the chlorophyll fluorescence kinetics. Basal Chlorophyll fluorescence increased above 44ºC in all wheat genotypes indication PSII damage due to temperature rise (**Brestic *et al*., 2013**). Studies apart from wheat under heat stress condition, through an experiment, 3cotton genotypes, TX2287, TX2285, TX761 has maintained high photosynthetic efficiency (high Fv/Fmax values) under elevated temperatures and considered as heat-tolerant genetic resource for improved upland cotton yield (**Su *et al*., 2014**). Seven winter wheat varieties were exposed to post-flowering heat stress using growth chambers [35/15 ◦C (heat stress) and 25/15 ◦C (control) day/night] and in field-condition heat tents (imposed+6◦Chigher than ambient). Quantum yield (QY) of photosystem II (PSII) was recorded in flag leaves and spikes. There was sustained heat stress induced accelerated decline in QY, particularly in wheat cultivars Larry andWB4458 that found susceptible towards heats stress. At the beginning of the experiment (time 0 and 10 days after flowering), the value of QY (∼0.7) was similar across flag leaves and spikes of all varieties in chambers and under field experiment this value was slightly lower (∼0.65). Further sudden declination in values of fv/fmax shows due to heat stress sand a positive correlation was established between number of days of senescence and QY values **(Šebela *et al*., 2020).** Four wheat genotypes were analyzed based on their heat sensitivity (heat-sensitive: LM19, SF1 and heat-tolerant: LM62, NS3) at their tillering stage. The plants were subjected to 4 treatments as control, drought, heat (36/26 ◦C day/night temperature) and combined drought+heat stress (36/26 ◦C day/night temperature with 40/60% day/night). No difference in Fv/fmax values was observed between the genotypes under control and drought treatment, may be due to mild heat stress experienced by genotypes and as a result of increased photorespiration, which maintains the electron transport rate and protects PSII from damage when stomatal limitation occurs. With heat stress, a significant decrement was observed in the values of Fv/Fm, with significantly lower ratios observed in NS3 compared to LM62. With combined stress, the Fv/Fm was also significantly lower in NS3 as compared to other wheat variety. The Fv/Fm was higher in the heat-tolerant compared to the heat-sensitive genotypes. During combined stress, the Fv/Fm was significantly higher in the heat-tolerant NS3 compared to LM62. Over all conclusion of the study is that the selected genotypes were very less (moderately) affected in their photosynthetic efficiency and growth under drought stress, whereas heat and combined stress caused rapid reductions in photosynthesis and growth in genotypes **(Abdelhakim *et al.*, 2021).**

2.5. Effect of elevated temperatures on biochemical parameters

2.5.1 Chlorophyll and carotenoids

Chlorophyll and carotenoids are the pigment molecule that plays essential role in photosynthesis. Light harvesting complex have different chlorophylls that traps the sunlight while carotenoid contributes to photosynthesis by transferring some of the absorbed light energy to chlorophyll molecule, which then use as energy to drive photosynthesis (**Tewari, 1990**). They also protect plants which are over exposed to sunlight by dissipating excess light as heat. From past researches, measurement of chlorophyll content & carotenoids could be useful for screening the heat tolerance trait in various crop species such as in wheat. As a result of delayed sowing in some crop species such as wheat, sorghum, cotton maize etc. results heat stress during their growth period responsible for disruption in photosynthesis process through disruption in structure and function of chloroplast and reduction in chlorophyll content (**Xu *et al.*, 1995**). The reason for inactivation of chloroplast enzyme under elevated temperature is mainly due to excess light and oxidative stress (induced by lipid peroxidation that cause protein degradation, membrane rupture and enzyme inactivation) led to decreased rate of leaf photosynthesis (**Sairam *et al.*, 2000**). Other enzymes such ribulose 1-5, bis phosphate carboxylase/oxygenase (RUBISCO) also found affected by elevated temperatures. As a result of high temperature, RUBISCO losses its affinity towards CO_2 (solubility of CO_2 decreased under elevated temperatures in air) and binds to O_2 which causes increase in photorespiration and decreased carboxylation hence, rate of photosynthesis decreases (**Long *et al*., 2004).** From previous studies a significant reduction in chlorophyll content and carotenoids was observed under heat stress due to heat induced damage to thylakoids. It was observed that protochlorophyllide oxidoreductase (POR) is greatly reduced under heat stressed wheat cultivars as compared to control shows a significant positive correlation between loss of chlorophyll and heat stress. A positive and linear correlation was found between rate of chlorophyll loss and photosynthate stem reserve (PSR) in wheat which indicates that under high temperature stress potential capacity of utilizing stem reserves for grain filling is accelerated but also accelerates leaf senescence (**Ristic *et al*., 2007).** In an experiment it was observed that both chlorophyll content and carotenoids of flag leaves in wheat decreased after anthesis and least chlorophyll content observed during grain filling stage (**Cai *et al*., 2008**). By various studies it is also concluded that heat tolerant wheat cultivar (Fang) had high chlorophyll content as well as carotenoids as compared to heat sensitive wheat cultivar (Siete Cerros) (**Tahir *et al*., 2009**). Six wheat cultivars (UP-2338, PBW-343, UP-2113, PBW-175, VL-616 and VL-421) were subjected to variable temperature conditions by a delay of 20 days in their sowing. A reduction in chla+b and chla/b ratio were observed under delayed sowing as compared to timely sown cultivars. The highest value (4.1 mg^{-1}g fw) of chlorophyll (a+b) during anthesis and chl a/b ratio (3.8) was observed in UP-2338undertimely sown conditions which declined to 1.5 and 1.6 respectively under late sown condition (**Srivastava *et al*., 2012)**. The elevated temperatures treatments up to 37ºC and 45ºC for 8hours on seedings of Karacadage and Firat wheat cultivars showed induced reduction in chlorophyll accumulation (**Iqbal *et al*., 2017**). According to previous studies, tolerant genotypes showed higher chlorophyll content and relatively lower reduction in values of chlorophyll under elevated temperature as compared to sensitive genotypes of wheat. The flag leaf chlorophyll content of different wheat genotypes was analyzed at 18 days after anthesis under normal, moderate and high temperature conditions. The results showed that flag leaf chlorophyll content was significantly influenced by the interaction effect of wheat genotypes and their growing conditions. Under normal growing condition, the wheat genotype BAW-1202 contained the highest flag leaf chlorophyll (1.63 mg g-1 FW). On the other hand, BARI Gom-27 had the lowest chlorophyll (0.94 mg g-1 FW) at 18 days after anthesis which was statistically similar to BARI Gom-26 (1.10 mg g-1 FW) and BAW-1182 (1.08 mg g-1 FW). Due to late sowing (warmer condition), the chlorophyll content reduced significantly in BAW-1202 but it was remained more or less unchanged in other three wheat genotypes (**Sharmin *et al*., 2020**).

In an investigation the effect of heat stress on carotenoid content of 11 wheat genotypes was evaluated, results had shown decreased carotenoid content in all the genotypes of wheat as compared to control. Under heat stress carotenoid was found within range of 0.0291-0.298 mg/g FW (**Kaur and Thid, 2017**). Carotenoids in plants responsible for various function such as photosynthetic accessory pigments, antioxidants, and vitamin ‘A’ precursors. In transgenic sweet potato the overexpression of *IbOr-R96H* leads to production of higher carotenoid content in abiotic stress condition that provide greater tolerance to heat stress (47 °C). During the experiment the total carotenoid contents in storage roots of sweet potato were 5.4–19.6 fold higher than non-transgenic sweet potato. These results indicates that *IbOr-R96H* is a promising strategy for developing new sweet potato cultivars with improved carotenoid contents and heat stress tolerance **(Kim *et al*., 2021).**

2.5.2 Proline content

Accumulation of organic compounds of lower molecular weight such as proline is a key adaptive mechanism of plants to cope up with biotic and abiotic stresses. It is major organic molecule that works as compatible osmolytes in stressed plant including extreme temperatures and involves in cytosolic osmotic adjustment during stress condition. Proline used as indicator of stress (stress related signal) (**Sakamoto and Murata, 2002)** as it scavenges hydroxyl free radicals and preserve protein structures and enzyme activity under stress conditions. Through various researches it is observed that proline accumulates in large amount in response to environmental stress and act as compatible solutes, thus proline can also be used as metabolic marker in relation to identification of thermo tolerant genotypes (**Arshi *et al*., 2005**). Proline enhances the stability of proteins and membrane under high temperature. During grain filling stage higher accumulation of proline in stressed leaves of wheat was studies this is due to the role of proline is related to protective action **(Iqbal *et al.*, 2017).** Proline content in flag leaves of different wheat genotypes was determined at 18 days after anthesis under normal, moderate and high temperature conditions and results showed that under normal condition, all 4 wheat genotypes contained more or less equal amount of proline (1.00 to 1.21 µmole g/FW) in flag leaf. With the delay in sowing i.e. with the elevation in temperature, the proline level in flag leaf was found increased. Under moderate high temperature condition, genotype BARI Gom-26 contained highest level of proline (2.09 µmole g/FW) which was statistically similar to BAW-1182 (1.91 µmole g/FW). While BARI Gom-27 contained the lowest (1.26 µmole g/FW) proline and BAW- 1202 contained the moderate (1.60 µmole g/ FW) amount of proline in flag leaf. Under high temperature, BARI Gom-27 contained highest proline (2.77 µmole g/FW) which was statistically similar to BAW-1182 (2.61 µmole g/FW) and BARI Gom-26 (2.61 µmole g/FW). While BAW-1202 contained the lowest proline (1.78 µmole g/ FW) (**Sharmin *et al*., 2020**). In an investigation, 17 wheat genotypes were sown under two sowing conditions with interval of 30 days. The results showed that high temperature stress due to late sowing causes accumulation of more proline content in flag leaved of wheat genotypes; NIA-Sunder, NIA-AS-14-10 and SRN-09111. Proline contents under normal sowing varied between 3 to 10 µmole g/Fwt. While, it raised to 4 to 5 times under late sown wheat genotypes. Maximum increase under late sown condition was observed for NIA-Sundar followed by NIA-AS-14-10 and SRN-09111.Highest proline accumulation under late sown condition is attributed to increase in γ-glutamyl kinase and decrease in proline oxidase activities. While heat sensitive genotypes failed to accumulate more proline as compared to tolerant genotypes **(Khan *et al.*, 2020).**

2.5.3 Nitrate Reductase activity

Nitrate reductase activity (NR actvity) plays important role in nitrogen metabolism in plants through reduction of nitrate to nitrite which is further reduced to ammonia. The reduction of nitrate to nitrite is catalyzed by nitrate reductase (NR) enzyme. Different wheat genotypes showed different ability to accumulate the reduced nitrogen during their growth and development (**Eilrich and Hageman, 1973)**. An experiment on Barley (Hordeum vulgare L.) leaves and spinach (Spinacia oleracea L.) related to chloroplasts exposure to short-term heat stress and its after effects on nitrate reductase activity were studied. The results showed that a gradual decrease in NR activity were observed from 37 to 40°C with increasing temperature. Enhanced mobilization of nitrate promotes the synthesis of nitrogen-containing organic substances, especially proteins, many of which are subjected to thermal denaturation, resulted reduction in NR activity (**Maevskaya, 2003**). According to previous reports, elevated temperature stress due to delayed sowing, global warming and climate change leads to reduces root growth, number, and mass in various crop species which affects the nutrient (N) uptake and nitrogen assimilation which ultimately reduce nitrate reductase activity in leaves (**Huang *et al*., 2012).** The effects of heat stress on root nutrient (N) uptake in *Solanum lycopersicum* (tomato) at 25 ◦C/20 ◦C (day/night), 35 ◦C /30 ◦C (moderate heat) and 42 ◦C/37 ◦C (severe heat) were evaluated. The results showed that the roots were found more affected than shoots which decreases the root:shoot mass ratio, shoot vs. root %N and C, and levels of nutrient-uptake and assimilation proteins in roots led to decrease in nitrogen assimilatory proteins; nitrate reductase and nitrite reductase **(Giri *et al*., 2017).** An experiment was performed to investigate the effect of high temperatures on wheat *(T. aestivium*) cultivars and it was found that NR activity was maximum during pre - flowering stage than post flowering in both cultivars. NR activity found higher in tolerant (C-306) than heat sensitive (HUW-468) (**Kumari and Hemantaranjan, 2019**). The effect of elevated temperature on nitrate reductase activity in *Ulva prolifera* (green algae) was observed under five different temperatures (10, 15, 20, 25 and 30°C) for 7 days. The results shows that the maximal NRA appeared with proper growth on the 2nd day in the 10, 15, and 20°C (low temperature) groups and on the 1st day in the 25 and 30°C (high temperature) group. While after 2nd day, the algal growth was decreased with decrease in its NR activity at a high temperature of 25 and 30 °C. After 5 days, *Ulva prolifera* cultivation was found with long growth cycle with 10-20°C but NRA decreased with increasing temperature when exceeded 15°C. A positive correlation between algal growth and NRA was also observed. This study supports that the NRA is a suitable marker for evaluation of the effects of temperature changes on the ability of *Ulva prolifera* to grow, uptake and metabolization of nitrogen nutrients (**Feng *et al.*, 2021).**

2.6 Effect of elevated temperatures on lipid peroxidation and detoxification system

Lipid peroxidation is an important feature of stress conditions. It refers to oxidative degradation of lipids i.e. free radicles that steal electrons from lipid cell membrane, resulting in cell damage. Oxidative stress induced by lipid peroxidation responsible for protein degradation, membrane rupture and enzyme inactivation led to decreased rate of leaf photosynthesis in various crop species, especially those are susceptible to elevated temperatures (**Sairam *et al.*, 2000**). Malondialdehyde assay, (MDA content) is an indicator of lipid peroxidation in the cells and used as marker of oxidative lipid injury caused by heat stress. According to some research, in late sowing condition for wheat, increasing temperatures significantly decreases relative water content and increases the level of lipid peroxidation (Malondieldehyde) and H_2O_2 content in all the wheat cultivar namely C306, HD2285, HD2329 in which those showed lower level of lipid peroxidation had higher degrees of heat tolerance (**Sairam *et al.*, 2004**). In an experiment, winter wheat (*T.aestivium* L. *cv*. Yangmai 16) were grown under four treatments; heat treatment (32/28°C for 2 days), non-heat treatment (24/20°C), heat treatment with no post anthesis high temperature treatment and non-heat treatment with post anthesis high temperature (34/30°C) treatment. The MDA content was analyzed in flag leaves of wheat under different treatments. The results revealed that the MDA concentration in wheat leaf was significantly increased after heat treatment (32/28°C for 2 days) & non-heat treatment (24/20°C) but showed no significant difference between heat treatment (32/28°C for 2 days) and non-treatment (24/20°C). While, non-heat treatment along with no post anthesis high temperature treatment shows higher MDA content than heat treatment (32/28°C for 2 days) along with post anthesis high temperature (34/30°C) treatment. This implies that photosynthetic apparatus found protected in heat treated plants than non-heat-treated plants which showed tolerant behavior of winter wheat cultivar “Yangmai 16” (**Xin *et al.*, 2016).** Two winter wheat varieties, Kraljica (normal sown + high yielding) and Olimpija (late sown+ high quality) were evaluated for their performance 0, 7, 14, 21, 28 and 35 days post-anthesis under similar sowing condition. The results showed that the Kraljica significantly increased MDA at the last sampling point, compared to Olimpija which shows that during grain filling (when temperature keeps on rising per day) the MDA content significantly increases in Olimpija as compared to Kraljica which can be used as an indicator of rapid senescence in Olimpija (**Spanic *et al*., 2020).**

Apart from mitochondria and peroxisomes, chloroplast (PSII and PSI reaction centers of thylakoid membrane) is considered as a significant source for production of various reactive oxygen species (ROS) under stress condition. It causes injury to plants in the form of oxidative stress leads to generation and reaction of activated oxygen species such as triplet stage of chlorophyll (Chl*), singlet oxygen ($^1O_2$), superoxide radicle (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). (**Liu and Huang, *et al*., 2000)**. As abiotic stress (heat stress) causes inhibition of transfer of excited energy in PSII antenna complex and electron transport in PSII reaction center resulting in formation of ROS in algae and higher plants. The production is also natural inside the cell but overproduction becomes harmful to plants (**Esfandiari., 2007**). Hydroxyl radicles can react to all biomolecules like pigments, proteins, lipids, DNA and almost all the constituents of cells singlet oxygen directly oxidize proteins, polyunsaturated fatty acids and DNA. Due to oxidative stress, decrease in photosynthetic light reaction was observed that led to increase in electron leakage from thylakoid membrane ((**Moller *et al*., 2007)**. In order to limit the oxidative stress in plants under stress condition there is a development of detoxification system that breaks down the highly toxic ROS into less toxic or harmless product. Plants produce various active oxygen radicles using antioxidant enzymes to protect cell and sub cellular system by cytosolic effect (**Almeselmani *et al*., 2009).** Studies suggested that ROS production in chloroplast can functions as plastids signal to in form the nucleus to activate the expression of genes encoding antioxidant enzyme and to adjust the stress responsive machinery for more adaptative to environmental stress. An antioxidant is a molecule capable of slowing or preventing the oxidation of another molecule. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cells. Antioxidant terminates this chain reaction. Inhibition of antioxidant enzymes causes oxidative stress (**Farooq *et al.,* 2011)**. The antioxidant defense mechanism is a part of heat stress adaptation and correlated with acquisition of thermotolerance. This antioxidant defense system includes enzymatic and non-enzymatic antioxidants. Some examples of enzymatic antioxidant system are ascorbate peroxidase, dehydro-ascorbate reductase, glutathione S-transferase, superoxide dismutase, catalase, guaiacol peroxidase and glutathione reductase while non- enzymatic antioxidants include glutathione, ascorbate and tocopherols (**Suzuki *et al.,* 2012**). During an investigation, two cultivars of *Brassica Campestris* L.,"wucai" (heat sensitive and heat tolerant) were evaluated under elevated temperatures. Sensitive cultivar showed severe damage to photosynthetic apparatus and membrane system under heat stress as more accumulated of ROS and MDA were observed in sensitive cultivar as compared to tolerant (**Zou *et al*., 2017).**

SOD catalyzes the breakdown of superoxide anion into oxygen and hydrogen peroxide. SOD contains metal ions as a cofactor so its isozyme can be copper, zinc, manganese or iron. According to some reports, SOD activity was found higher in tolerant wheat cultivar in comparison to susceptible cultivars. SOD activity also found to be increased in case of late sowing and very late plantings. Highest SOD activity was recorded at 15 days after anthesis in very late planting followed by anthesis stage and lowest activity was recorded at vegetative stage (**Almenselmania *et al*., 2009**). Further hydrogen peroxide in plants is taken care via catalase and peroxidase. Both guaiacol peroxidase and ascorbate peroxidase can also detoxify hydrogen peroxide but they require hydrogen peroxide to be scavenged by reducing agent (**Goyal and Asthir, 2010).** An experiment was done to evaluate the effect of terminal heat stress on superoxide dis mutase (SOD) activity in wheat variety (Faisalabad-2008) with treatments; control, terminal drought stress alone (50% field capacity during reproductive phase), terminal heat stress alone (wheat grown inside plastic tunnel during reproductive phase) and terminal drought stress + terminal heat stress. The SOD activity was found significantly increased with exposure of stresses and the highest SOD activity (54%) was observed under combined stress condition. As ROS generation due to heat stress induces the production of abscisic acid that act as a signal molecule under stressed conditions and regulate the gene expressions that control the production of enzymatic antioxidants such as superoxide dismutase (**Sattar *et al.*, 2020**). A new potential biochemical marker (Mn-SOD) gene of ~733 nucleotide long is identified from wheat *cv*. HD2985. The location of the gene is on Chromosome 6D. Maximum expression and activity of Mn-SOD was observed in leaves of wheat *cv*. Raj3765 in late grain filling stage as compared to stem and spike under heat stress condition that shows Mn-SOD is directly involved in thermotolerance in wheat (**Kumar *et al.*, 2020**).

CAT activity catalyzes the decomposition of hydrogen peroxide to oxygen. Heat treatment increased the CAT activity in T. *aestivum* genotype whereas, decreased in T. durum genotype of wheat (**Keles and Oncel., 2002**). During an experiment it was investigated that there was high antioxidant potential in thermo-tolerant wheat genotype as compared to thermo-susceptible.During pollination a significant increase in CAT activity was observed in Halna (thermo-tolerant) in response to T1 (30 ºC) treatment (15.8 µg/ mg protein) and T2 (38°C) treatment (16.4 µg/ mg protein) as compared to other cultivar, CAT activity was found very low in HD 2329 (thermo-susceptible under T1 (8.12 µg/ mg protein) and T2 treatment (8.65 µg/ mg protein) (**Kumar *et al.*, 2018**). A wheat variety was subjected to various combinations of heat treatment in comparison to control led to conclude that under elevated temperature catalase activity in plant was increased (61%) to compensate the bad effect of ROS and convert harmful active species to inactive condition. (**Sattar *et al.*, 2020**). An experiment was done to evaluate the biochemical responses in contrasting thermo-tolerant BARI Gom 26 ("BG26") and heat susceptible Pavon 76 ("Pavon") wheat varieties when they were exposed to 25°C (control) and 35°C (heat stress), stress condition during the seedling stage. The results showed that the tolerant variety maintained its thermo-stability under heat stress condition by accumulation more antioxidant activity or least reduction in CAT activity (15 to 20%) as compared to the heat- susceptible where the reduction in CAT activity due to heat stress was found 38% in comparison to control (**Mohi-ud-Din *et al.*, 2021**).

2.7 Effect of elevated temperatures on grain quality

Grain quality is a range of physical and compositional properties for any cropwhere threshold values are set according to nutritional requirements. Now a days, a uniform grain quality is very difficult to achieve as changing climate and elevated temperatures causes irreversible damage to different grain component toa different extent (**Castro *et al.*, 2007**). So, it is very important to know the extent of changing grain components in respect to unfavorable climate conditions and elevated temperatures. According to previous studies the ultra-structure of bread and durum wheat grains under heat stress condition during grain filling was studies which showed that the heat stress causes triggered grain shrinkage with reduced weight. Changes in aleurone layer as well as in endosperm cells was also observed. Heat stress affects the gluten strength of wheat cultivar was also found affected that responsible for diminishing the wheat flour quality (**Dias *et al.*, 2008**). Physical properties such as grain size and shape in wheat is also influence the milling yield as if grain is small and shriveled it reduces milling yield by 2% i.e. the loss of milling yield of 7$ per tons (**FAOSTAT, 2014)**. Wheat is an annual, long day and self-pollinated plant whose quality characteristics such as flour protein concentration, milling yield, rheological properties and bread making properties are highly influenced by Genotype, Genotype×Environment interactions which results great shift in overall performance of crop and its quality characteristics (**Dia *et al.*, 2016**). Grain quality data can also be evaluated on the basis of carbohydrates, starch, amylose, grain nitrogen, protein and storage protein in them. Detail view on the effect of elevated temperatures due to delayed sowing and climate variations on various grain quality parameters are described below;

Carbohydrates are known to be the major component of assimilation flux in filling grains. According to researches, carbohydrate metabolism pathway such as sugar conversions, glycolysis, TCA cycle and lipid metabolism were decreased in response to heat stress (**Zahedi *et al.*, 2003**). Sucrose is the main photosynthetic product of leaves and key enzyme sucrose synthase (SS) necessary for sucrose to enter into various metabolic pathways, SS plays important role in plants growth and development. From an experiment, in which winter wheat cultivar Gaocheng8901 was grown under two different sowing conditions one as a controlled (25ºC/18ºC day and night temperatures) and another as a treatment of (40ºC for 2 hours from flowering to maturity). The expression of SS was found reduced under heat stress condition indirectly inhibiting carbohydrate synthesis and thus reducing wheat yield (**Zhang *et al.*, 2018**). According to a study in 237 spring wheat varieties under different sowing months (October) & (February) as normal and delayed condition, the metabolomic analysis demonstrated the decrease in 16 out of 21 metabolites related to carbohydrate metabolism pathway under elevated temperature due to late sowing. As carbohydrates are well known major component of assimilation flux in filling grains, but due to elevated temperature led to less assimilation allocated to grains causes less carbohydrate content in grains However, G1P (Glucose 1 Phosphate) and G6P (Glucose 6 Phosphate), the carbohydrate precursor for starch synthesis remains stable in heat stress which is found responsible for stable filling rate during late sowing condition (**Wang *et al.*, 2018**).

Starch content in wheat kernel accounts almost 70% of total grain dry weight and reduced starch deposition under high temperature is the main reason for reduction in grain weight under elevated temperature (**Bhullar and Jenner, 1985**). Reduction in starch deposition in grains is mainly due to the enzymes which are required for starch biosynthesis (Sucrose synthase, soluble starch synthase and granule bound starch synthase) are found sensitive towards temperature rise from 18 to 22ºC. According to one research, temperature above 18 to 22ºC lead to reduction in grain dry matter as reduction in starch formation observed (**Hawker and Jenner, 1993**). In wheat, the Rate of starch synthase determined by Sucrose synthase whereas, granule bound starch synthase enzyme controls amylose biosynthesis (**Morell *et al.*, 2001**). Soluble starch synthase (SSS) also regulates starch synthesis and highly sensitive to heats stress. Heat stress decreases the activity of Soluble starch synthase which reduces grain growth and starch accumulation. Even short period of temperature fluctuation results in degradation of enzyme SSS (**Prakash *et al.*, 2003**). Stable grain starch content was observed in Hindi62 (heat tolerant) whereas, in PBW154 (heat sensitive wheat cultivar) there was reduction in starch content under heat stress. Similarly, during grain development in wheat, heats stress badly affects the starch content, causes poor grain quality, grain size and yield (**Chinnusamy and Chopra, 2003**). The influence of high temperature on quality of starch was studies in bread, biscuit and durum wheat for two seasons and it was concluded that there is reduction of weight and diameter of kernels, thus reducing starch content to significant level (**Labschage *et al*., 2009**). The link between temperature during the grain filling phase and starch granule deposition provides opportunity to identify starch granule size distribution and also make estimate of emergent properties such as grain milling, starch digestibility and dough visco-elastic proteins in wheat (**Nuttall *et al*., 2017).** An experiment was done to analyze the thermo-tolerance in 237 spring wheat varieties under two different sowing months; timely sown (October) and late sown (February). Results conclude that the starch accumulation which is a major component of deposition in grains found reduced during heat stress this is due to decrease in 3 starch synthesis related protein (two starch synthase and granule bound starch synthetase) under delayed sowing condition (**Wang *et al*., 2018).** An experiment in maize, was done to investigation the seed setting of kernels under different abiotic stress condition drought, heat and combined drought and heat stress (DS, HS and DHS) with two maize hybrids. The result showed that the starch content in the kernel was reduced by 42 % in drought treatment, 29 % in heat stress treatment and 58 % in combined treatment. Some of the reasons for the decrement in starch content explained by investigator may be due to limited assimilates under stress condition or sugar-to-starch synthesis was disturbed under stress condition **(Ayub *et al*., 2021).**

Starch synthesis constitutes two classes of polymers, amylose (25%) and amylopectin (75%), which are straight chained and highly branched molecules respectively. Amylose is the linear polymer of glucose molecule with α-1, 4 linkages while amylopectin is branched polymer with the α-1, 6 linkage. The synthesis of amylose occurs in non-green part of plants and enzyme; granule-bound starch synthase (GBSS) involved in amylose synthesis. Under heat stress during grain filling (>30 ◦C) the amylose/ amylopectin ratio increases which causes a reduction in dough elasticity. The size distribution of starch granules is also modified by high temperatures (**Hurkman *et al*., 2003**). Due to heat stress, an increase in the degradative isoenzymes (α/β-amylases) was observed in at 38 °C, 2h treatment in wheat HD2329 (thermo-susceptible) **(Kumar *et al.* 2018).** An experiment was done with two wheat cultivars; HD3059 (thermotolerant) and BT-Schomburgk (thermo-susceptible) at grain-filling stage under heat stress treatments of 32 °C and 40 °C for 1 h. The results conclude that both the cultivars showed decrease of 48-58% in starch synthase activity and starch content under heat stress condition. The reason for the decrement is that the enzymes associated with starch biosynthesis are heat sensitive and an increase in the temperature adversely affects starch synthase (SS) activity **(Yang *et al.* 2018).** On comparing the wheat genotypes, starch synthase and starch content were found higher in HD3059 (thermo-tolerant) as comparedto BT-Schomburgk (thermo-susceptible). While amylose content was found higher under elevated temperature, as increase in temperature lead to disturb the amylose/amylopectin ratio hence amylose found higher under heat stress condition. On comparing with the wheat genotypes higher value of amylose content was observed in BT-Schomburgk (12%) (**Kumari *et al.*, 2020**).

Grain nitrogen (N) is also a key quality parameter that defining nutritional and functional properties of cereals. Daily crop nitrogen uptake is usually defined by the minimum of nitrogen demand and potential uptake its transfer to grain (**Palta and Fillery, 1994**). Nitrogen requirement for protein synthesis during a developing kernel is done by the mobilization of previously assimilated nitrogen present in reserved in vegetative tissues along with the direct uptake and assimilation of nitrogen at the time of grain filling. Mobilization of previously assimilated nitrogen is considered as a major source of nitrogen for protein synthesis in kernel filling (**Austin *et al.*, 1977**). About 60 to 95% of grain N comes from the remobilization of N stored in roots and shoots before anthesis. Previous reports suggested that during grain filling of wheat, N accumulated in the vegetative organs is also found remobilized to the ear (**Triboi and Triboi-Blondel, 2002).** Under heat stress protein synthesis in cereal crops reduced but storage protein (gliadins) and heat shock proteins increase as result nitrogen content per storage protein also found to be increased (**Trebst e*t al.*, 2002**). When the total amount of nitrogen in grains increases the glutein fraction remains stable while gliadins fraction increases. Gliadins known to confer extensibility to the dough.

Reports also concluded that during heat stress grain filling duration decreases, it affects the grain dry matter and nitrogen accumulation. So as the grain nitrogen increases, with increase in Gliadin's content the dough making property of wheat affected (**Nuttall *et al*., 2017**).

Mature wheat grain contains 8 to 20% of protein. The ability in wheat flour to be processed into different food items is determined by its protein concentration. Under elevated temperature grain protein percentage was enhanced while the protein content per grain was reduced (**Krishnan *et al*., 1989).** When wheat coleoptiles expose to high temperature stress results in synthesis of group of proteins known as heat shock proteins. These proteins (Heat Shock Proteins; HSPs) were found different in comparison to normal proteins and provide thermo-tolerance. They are lower molecular weight proteins and have thermo-tolerant characteristics in them (**Blumenthal *et al*., 1991)**. A significance of heat stress response and expression of HSPs in thermo-tolerance of cereal yield (wheat) and quality has been studied, on the bases of this study it is concluded that the high temperature during grain filling activates heat shock genes causing the mature grain to contain more protein but this accumulated protein provide thermo-tolerance to grain & produce weaker dough. Heat shock proteins synthesized at 34°C and disappeared after shifting of seedlings to 22ºC. The synthesis of HSPs was responsible for acquisition of thermo-tolerance for seedling to survive (**Rampino *et al*., 2009**). Previous reports suggested that accumulation of more soluble proteins under heat stress is considered as another reserve in grains (~20%) which is responsible for compensate for reduction in starch deposition and hence to maintain stable filling rate, as assessed by weight changes **Farooq *et al*., 2011).** In an experiment, *Chinese spring* wheat kernel was evaluated under heat treatment in comparison to control condition it was found that under heat stress condition a general decrease in protein synthesis components and metabolic proteins (sucrose and starch synthesis proteins is observed but a significant increase in stress responsive protein (HSP70, HSP40, HSP20, redox responsive proteins) and storage protein is observed which leads to increase in total protein content in wheat kernel under heat stress condition. Metabolomic study suggested that, less energy (ATP) was channeled into metabolism and protein synthesis, whereas more energy was allocated to stress responses under elevated temperature conditions (**Wang *et al*., 2018**). A significant increase in total protein (11.5 to 13.5%) content in grains of 17 Indian wheat genotypes was reported when they sow late (12th December 2016) as compared to normal (15th November). While highest increase in protein content was observed in variety CL3949 48% due to delayed sowing **(Singh *et al*., 2021).**

Wheat protein have high complexity and various types of interactions in it; thus, it makes the protein difficult for characterization. On the basis of the solubility and by following the sequential Osborne extraction procedure, total wheat soluble protein is divided into 4 classes; albumins (water soluble), globulins (salt soluble), gliadins (alcohol soluble) and glutenins (Acid soluble) (**Kirlman *et al*., 1982).** Albumins and globulins (non-glutene) represent 20 to 25% of total grain protein. They have good amino acid balance so they are important as nutritional point of view. Wheat flour has unique, complex protein called gluten that have property to form dough. This gluten is responsible for the rheological property of wheat flour which is important for bread making along with other food stuff such as noodles, pasta, cookies and other products **(Shewry *et al*., 1986**). The gluten protein consists monomeric gliadins and polymeric glutenin. They are considered as major wheat storage protein which constitutes about 75-85% of total protein with ratio of 1:1 in common or bread wheat. They are also rich in asparagine, glutamine, arginine or proline ((**Shwery *et al*., 2007)** but very low in nutritionally important amino acid (methionine, tryptophan, lysine**) (Zilic *et al*., 2011**). According to studies a great increase in grain protein was observed when the wheat cultivar experience heat stress during early grain filling stage. However, exposure of heat stress decreases synthesis of glutein while synthesis of gliadins remains constant/increases in some cases (**Majoul *et al*., 2003**). Heat stress also decrease sedimentation index as an effect associated with increasing protein content in grains but decrease with essential amino acid. During an experiment in wheat genotypes (Sever, Golia, TE 9306 and Acalo) that were subjected to temperature treatments of (31/20ºC) and control of (25/14ºC) after anthesis. The results showed grain shrinkage, reduced grain weight (upto 17%) and changes in ultrastructural of aleurone layer and in the endosperm cells in all wheat genotypes under heat stress condition as compared to controlled. Heat stress also causes decreased sedimentation index along with increased protein content (upto 40%) in the grains but levels of essential amino acids were found decreased (upto 30%). Over all reduced nutritive value was observed under elevated temperatures as compared to normal (**Dias *et al*., 2008**). In an experiment, winter wheat cultivar Gaocheng 8901 was grown as controlled (25°C/18°C). Heat treated condition (40°C for 2h) was given in growth chamber from flowering to maturity, the results show that under heat stress condition, grains become narrow, reduced weight while length remain unchanged. Protein concentration was found increased along with significant increment in soluble storage protein (gluten, gliadin, globulin and albumin) (**Zang *et al*., 2018**). In a study, wheat variety "Rete nazionaleduro" were grown in 4 locations having different temperature variations. The synthesis of gluten and gliadian proteins were evaluated from about 8 to 35 days after anthesis. The results shows that higher temperature during grain filling, causes increase in low molecular weight glutenins, in the glutenin/gliadin ratio and in the A-type starch granules sizeas the wheat cultivar tends to complete its anthesis and approaches towards grain filling. The results also conclude that as temperature increases, gliadins being accumulated faster than glutenins due to which glutenin/gliadin (glu/gli) ratio decreases progressively during grain development. Over all conclusion of study is that the protein content may vary due to stressful environmental conditions during grain filling, which act upon both kernel size and composition hence affect quality parameters (**Graziano *et al*., 2020).**

Materials and Methods

Chapter 3 MATERIALS AND METHODS

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

3.1 EXPERIMENTAL STIE

The present research was carried out during Rabi seasons 2018-19 and 2019-20 at Dr. N.E. Borlaug Crop research center (CRC), G.B Pant University of Agriculture & Technology, Pantnagar, Udhan singh Nagar (Uttarakhand) India. Geographically, research site lies in Tarai plains about 30 km southwards of foothills of Shivalik range of Himalayas at 29º N latitude, 79º 29'E longitude and at an altitude of 243.84 meter above sea level.

3.2 CLIMATE AND WEATHER CONDITIONS

The climate in Tarai region (Pantnagar) is humid subtropical with hot and dry summers and cool winters. Winter season extended from November to March. The monsoon sets during 2nd or 3rd week of June and continuous till September end. Winters may or may not receive few showers. Weather parameters such as minimum and maximum temperature, relative humidity and rainfall during the period of research, recorded at meteorological observatory located at crop research center, pantnagar, are presented in (**Appendix VII**).

3.3 INSTRUMENTS USED FOR THE EXPERIMENT

- Digital Balance Citizen Balance, Solan, India
- Digital pH Meter GENEI, Bangalore
- Micropipette Nichipet (Japan); Tarson (India)
- Microtips Tarsons
- Eppendrof Tarsons
- Refrigerator LG, Godrej (India)
- Deep Freezer Vestfrost
- Centrifuge Sigma 3 – 18K (USA)

- Centrifuge Tube Tarsons
- Water Bath Narang Scientific works, India
- Hot Air Oven Macro Scientific works, India
- U.V- Visible Spectrophotometer Thermo Fisher Scientific (U.S.A)
- Kjeldhal apparatus Pelica (India)
- Handy Pea meter Hansatech, UK
- Gel Casting Assembly GENEI, INDIA
- Conductivity Meter Hanna Instruments
- Vortex Genei Banglore
- Telatemp infra-red thermometer (Model AG-42)
- Magnetic Stirrer Remi, Instrument Ltd. India

3.4 EXPERIMENTAL MATERIAL

The seeds of 8 wheat varieties namely were used as experimental material and were provided by the Department of Genetics and Plant breeding. College of Agriculture, G.B Pant University Pantnagar.

S.No.	VARIETIES	CHARACTERISTICS
1.	UP2628	Recommended for timely sowing
2.	HD3086	Recommended for timely sowing
3.	UP2967	Recommended for timely sowing
4.	UP2784	Recommended for timely sowing
5.	UP2526	Recommended for late sowing
6.	UP2565	Recommended for late sowing
7.	UP2748	Recommended for late sowing
8.	HD3059	Recommended for late sowing

3.5 CHEMICALS AND GLASSWARES

Chemicals used in the experiment were obtained from E. Merck (India) Ltd., Spectrochem (India) Ltd., Sigma Aldrich (USA) and Hi- Media Lab Pvt. Ltd. (India). Glass wares used in the study were obtained from Borosil, Bombay, India.

3.6 EXPERMENTAL DESIGN AND LAYOUT

The field experiment was conducted during two consecutive winter seasons comprising two sowing dates in November (D1) and December (D2) with 8 wheat varieties. All varieties were grown under randomized block design with three replications each. The replications were separated from each other with the help of proper bunds. Three plants were randomly selected for purpose of morpho-physio and biochemical studies.

Representation of zic-zac arranged wheat varieties under both sowing conditions

3rd Replication	V17	V18	V19	V20	V21	V22	V23	V24
2nd Replication	V16	V15	V14	V13	V12	V11	V10	V9
1st Replication	V1	V2	V3	V4	V5	V6	V7	V8

Note: V1 to V8 (**1st *Replication***) represents varieties in serial order mentioned above in "*Experimental material" section*. V9 to V16 (**2nd *Replication***) represents varieties order similar to V1 to V8. Similarly, V17 to V24 (**3rd *Replication***) represents varieties order similar to V1 to V8.

3.7 EXPERIMENT DETAILS:

Experimental Design: Randomized Block Design

No. of Treatments: November sowing (D1) and December sowing (D2).

1st Trial: November 20, 2018 and December 22, 2018 (E3 and E4 block of C.R.C)

2nd Trial: November 25, 2019 and December 28, 2019 (E3 and E4 block of C.R.C)

Number of varieties: 8

Number of replications 3

Total plot size: 4 rows × 3 meters (for each replicate)

Plant spacing (row to row): for November sowing (20 cm), for December (18 cm)

Cultural Operations

Seed bed preparation

After harvesting of previous crop, the field was prepared by deep ploughing using tractor drown soil turning plough followed by two cross harrowing and leveling. In order to create ideal sowing conditions for seed germination, pre sowing irrigation was given. For sowing, about 3 cm deep furrows were manually opened 30 cm apart and each furrow was uniformly sown with pre weighed seeds, 30 grams per packet (for November sowing) and 35 grams per packet (for December sowing) and covered immediately.

Sowing

All wheat seeds were sown using seed rate of 100kg/ha of the varieties and according to the dates mentioned for November and December sowing conditions. Seeds were treated with fungicide (Vitavax@ 2.0g/kg seed).

Fertilizer application

The crop was fertilized with Nitrogen @ 180kg/ha, Phosphorus @ 60 kg/ha and Potassium @ 40 kg/ha through urea and single super phosphate in both years. Half dose of nitrogen and full dose of phosphorous was applied as basal and remaining half dose of nitrogen was applied one day before first irrigation i.e: 25-30 days after sowing.

Irrigation and weed control

Irrigation was provided whenever necessary particularly during all the critical physiological growth stages of crop. Weeds are removed manually with *khrupi* whenever required.

Harvesting and threshing

The crop of the experiment sown on different dates was harvested at proper stage of maturity. The plot of each replication individually harvested manually with the help of sickle and the produce was tied in to a bundle and tagged. The bundles were allowed to dry in sun for few days. After weighing, threshing of bundles was done by Pullman thresher.

3.8 OBSERVATIONS RECORDED AND PROTOCOL USED

The observations were recorded during growth and development of crops. Biochemical analysis was done in laboratory.

Morphological Characteristics;

a) Plant height

Plant height of different wheat varieties were observed in centimeters (cm) by taking average height of three randomly selected plants from base of soil up to the tallest leaf tip. It was measured with the help of measuring scale at the time of maturity.

b) Number of tillers and productive tillers per plant

Tiller number of different wheat varieties were observed by counting tillers of three randomly selected plant of each variety from each replication at the time of anthesis.

C) Internodal distance

Internodal distance were recorded from top to base (1st internode, 2nd inter node and 3rd inter node) of three randomly selected plant of each variety from each replication at the time near to maturity.

d) Flag leaf length and width and area

The flag leaf length and width were recorded with the help of meter scale in centimeter. Final flag leaf length was the average of length of 3 randomly selected flag leaves from each 3 replication of each sowing conditions for each wheat varieties during anthesis stages and final flag leaf width was the average of width of the 3 randomly selected flag leaves of the same. Area was calculated by formula given by (**Gardner *et al.*, 1985**)

$$\text{Area (cm)}^2 = \text{length} \times \text{width} \times \text{correction factor } (0.75)$$

e) Leaf Area Index (LAI)

The leaf area index per plant was recorded at the time of anthesis by taking three replicates from both sowing conditions for each wheat genotype. The number of large, medium and small leaves were separated and length & width of leaves was also recorded by meter scale from single hill. LAI was calculated with the help of formula given (**Yoshida, 1981**).

$$LAI = \frac{(Area \times Number\ of\ leaves\ per\ hill)}{200}$$

$$Area = \frac{(L1\times W1\times CF)+(L2\times W2\times CF)+(L3\times W3\times CF)}{3}$$

$$Final\ LAI\ of\ variety = \frac{(LAI\ 1+LAI\ 2+LAI\ 3)}{3}$$

Where,

L_1 = average length of 3 large leaves of hill, W_1 = average width of 3 large leaves of hill

L_2 = average length of 3 medium leaves of hill, W_2 = average width of 3 medium leaves of hill

L_3 = average length of 3 small leaves of hill, W_3 = average width of 3 small leaves of hill

CF = correction factor (0.75)

f) Flag leaf fresh weight and dry weight

For measurement of fresh weight, 3 flag leaf at the time of anthesis from each mother shoot of each replication were harvested, washed with distilled water and blotted with blotting paper to remove excess water. The fresh weight was recorded from leaves and expressed in grams. Subsequently, flag leaf was kept for drying in hot air oven at 60ºC for 48-50 hours and then dry weight was monitored and expressed in grams.

g) Specific leaf weight (SLW)

Specific leaf weight was determined at the time of anthesis by collecting flag leaf from each replication. The leaves were oven dried at 60ºC for 48-50 hours and dry weight was taken. Before that their length and width were also measured for area calculation.

$$SLW\ (mg/cm^2) = \frac{Dry\ weight}{leaf\ area}$$

h) Total dry matter

The plant samples were collected during anthesis and maturity stage in replications and kept in paper bags and then dried in an oven for 3 days at 65 ºC. Dried weight of samples was measured with the help of weighing balance and expressed in grams per plant.

PHYSIOLOGICAL CHARACTERS

a) Relative water content (RWC)

Relative water content of wheat flag leaves was determined at anthesis and 15 DAA (Days after anthesis) by the method described by **Slatyer and Barrs (1965).** Three flag leaves were detached randomly from each plot. The leaves were cut into 3-4 cm long pieces and their fresh weight (FW) was taken immediately. Then the leaves were treated with distilled water in a petri plate for more than 8 hours. The leaf pieces were taken out and by using blotting paper, the excess water on the surfaces was removed. Then leaves were weighted and turgid weight (TW) was recorded. Then leaves were oven dried at 65-70º C for 2 days and dry weight (DW) was recorded. The RWC was calculated by using formula,

$$RWC\ (\%) = \frac{FW-DW}{TW-DW} \times 100$$

b) Membrane stability Index

Membrane stability index of wheat flag leaves was examined at the time of anthesis and 15 DAA according to (**Sairam *et al.,* 1997).** 0.5 grams leaf discs from both sowing condition and from each replication were taken in 25ml beaker containing 20ml distilled water in two sets. One set is kept at 40º C for 30 minutes and another set at 100 º C for 1 hour in a boiling water bath. Their respective electrical conductivity i.e. C1 and C2 were measured with a conductivity meter. MSI calculate by applying formula,

$$MSI\ (\%) = \left[1- \frac{C1}{C2}\right] \times 100$$

c) Chlorophyll ‘a’ fluorescence variable yield (Fv/Fmax)

Chlorophyll fluorescence of wheat flag leaves was measured at anthesis and 15 DAA with the help of Handy PEA (Hansatech, UK). Dark adaptation of samples (leaf) was achieved via light weight plastic leaf clips, the leaf clip shutter blade was closed to prevent entry of light and clip left in place for 10 minutes to provide dark adaptation. To perform a measurement, the Handy PEA sensor unit was held over the clip holding leaf middle part and the shutter opened. A single button presses immediately displaying automatically calculated fluorescence parameter quantum yield ‘Q’ or Fv/Fmax. All observations were recorded in forenoon hours 9 to 10 am to avoid photoinhibition.

F0 = Fluorescence level when plastoquinone electron acceptor pool (a_a) is fully oxidized.

Fm = Fluorescence level when is transiently fully reduced.

Fv = Variable fluorescence (Fm-F0)

d) Canopy temperature depression (CTD)

Measurement of canopy temperature depression (CTD) was taken at the time of anthesis and 15 DAA with the help of Telatemp infrared thermometer (Model AG-42) as described by **(Hatfield, 1979).** The IR thermometer was positioned one meter above canopy at 45° inclination and canopy temperature recorded between 11am to 1 pm (mid-day), when practically no wind velocity was there. Then CTD calculated by using formula,

CTD = Air temperature (ºC) – Canopy temperature (ºC)

BIOCHEMICAL ANTIOXIDANTS PARAMETERS

a) Chlorophyll and Carotenoid Content

Chlorophyll and carotenoid content in flag leaves of wheat varieties under both sowing condition with replications was estimated according to (**Hiscox and Isealesham 1979)** during anthesis and 15 DAA. In each test-tube 10ml of Dimethyl sulfoxide (DMSO) and 50 mg leaf sample were taken. The test-tubes then incubated at 65ºC for 3 hours in oven. The content was shaken twice during incubation time. After 3 hours, OD was taken at 480nm, 649.1nm and 665.1nm by using a multi-wave length spectrophotometer against pure DMSO as blank. The chlorophyll and carotenoids content were calculated by using equation given by (**Wellburn, 1994**).

Equations:

Chlorophyll 'a' (mg/g FW) = $\frac{(12.47\times A665.1-3.62\times A649.1)\times Volume\ of\ DMSO\ used(ml)}{1000\times Wt\ of\ sample\ (g)}$

Chlorophyll 'b' (mg/g FW) = $\frac{(25.06\times A649.1-6.5\times A665.1)\times Volume\ of\ DMSO\ used\ (ml)}{1000\times Wt\ of\ sample\ (g)}$

Total Chlorophyll (mg/g FW) = *Chlorophyll 'a' (mg/g FW) + Chlorophyll 'b' (mg/g FW)*

Carotenoid Content (mg/g FW) = $\frac{\left[\frac{1000\times A480-1.29\times\ chl\ a-53.78\times chl\ b}{220}\right]\times Volume\ of\ DMSO\ used\ (ml)}{1000\times Wt\ of\ Sample\ (g)}$

b) Proline Content

Proline in wheat flag leaves of each replicate under both sowing conditions was estimated according to the procedure of **Bates *et al.*, 1973** during anthesis and 15 DAA. 0.5g of flag leaf sample homogenized in 5ml of 3% aqueous sulpho-salicylic acid. The homogenate then filtered with Whatman No.2 filter paper. 2 ml of filtrate taken in fresh test tube and 2ml of glacial acetic acid and 2ml of acid ninhydrin (prepared by taking 1.25g ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid) were added to it. The reaction mixture then heated in boiling water bath for 1 hour. After 1hour, the ongoing reaction was terminated by placing the tubes in ice bath. Then 4 ml of toluene added to the reaction mixture and stirred well for 20-30 seconds. The pink colour aqueous phase was separated and warmed at room temperature and absorbance recorded at 520 nm by using toluene as a blank.

For standard curve preparation, standard proline solution ranging from 0, 5 10, 15, 20, 25, 30, 35 and 40 µg/ml were taken in 9 separate test tubes and final volume made-up to 2ml with help of 3% sulpho-salicylic acid. Rest of the procedure as same as above. Amount of proline in test samples was calculated through standard graph and expressed as proline content on fresh weight- basis as follows,

Proline (µ moles per gram tissue) = $\frac{\mu g\ Proline\ per\ ml\times ml\ toluene}{115.5}\times\frac{5}{0.5(grams\ sample)}$

c) Nitrate reductase activity

The nitrate reductase activity (NR activity) was estimated *in vivo* in freshly plucked leaves during anthesis and 15 DAA by using method described by **Haageman and Hucklesby (1971).** 0.5 grams fresh leaves of each sample were chopped finely and taken in test tube in duplicates and 3 ml of KNO_3 (0.4M) and 3 ml of phosphate buffer (0.3M) added and test tubes kept in ice bucket for 2 minutes. Then one set of test tube incubated in water bath at 30 ºC for 10 minutes ad another set at 30ºC for 40 minutes. 0.2 ml sample solution taken in two separate test tubes and 1.8 ml of distilled water added to it. Then 2ml mixture of 0.02 % NEED and 1% Sulfanilamide in ratio 1:1 was added. The test tubes kept in dark to develop pink color and OD was taken at 540 nm against blank (prepared by distilled water in place of sample). The amount of nitrate reductase activity produced by g fresh weight of sample was calculated by the following formula,

$$\mu\ mol\ NO2-\ /g\ fresh\ weight\ at\ 10\ min = \frac{Ab\ 540\ nm(10\ min\ sample)\ \times 0.081\times 26\times 1}{0.5}$$

$$\mu\ mol\ NO2-\ /g\ fresh\ weight\ at\ 40\ min = \frac{Ab\ 540\ nm(40\ min\ sample)\ \times 0.081\times\ 25.8\times 1}{0.5}$$

$$NR\ enzyme\ activity\ (\mu\ mol\ NO^{2-}/g\ fresh\ weight\ h^{-1}) = 2(nitrate\ produced\ at\ 40\ min - nitrate\ produced\ at\ 10\ min)$$

d) Malondialdehyde (MDA) Assay

The lipid peroxidation in flag leaves due to stress condition was estimated by measuring the amount of MDA content during anthesis and 15 DAA by the method described by **Health and Packer, 1968** which takes into account the possible influence of interfering compounds in the assay for Thiobarbutaric acid (TBA) reactive substance. 0.2 grams of fresh flag leaves from each replication were taken and homogenize with 2 ml of TBA (0.25% TBA in 10 % TCA (Trichloro Acetic Acid). The homogenate was heated at 95ºC for 30 minutes and cooled rapidly in ice bucket. The content was centrifuged at 10,000g for 30 minutes and supernatant was collected. The absorbance was recorded at 532 nm and 600 nm against blank containing 0.25% TBA in 10 % TCA. The concentration of MDA content was calculated by using following formula with extinction coefficient of 155mM^{-1} cm-^{1} .

$$\text{MDA content } (\mu mole/g \text{ FW}) = \frac{(Abs\ 532 - Abs\ 600nm)}{155} \times 1000 \times 2 \text{ (Volume of supernatant collected)}$$

e) Superoxide dis-mutase (SOD) activity

Superoxide dismutase was assayed in flag leaves of different wheat varieties of each replication during anthesis and 15 DAA by using procedure given by (**Beauhamp and fridovich, 1971**). 0.2 g of flag leaf samples were crushed in 2 ml of 0.1 M potassium phosphate buffer of pH 7, centrifuged at 16000g for 15 minutes under 4 ºC. The supernatant was collected and used as enzyme extract. For 3ml reaction cocktail containing 50mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 0.1 µM EDTA, 75 µM Nitroblue tetrazolium and 50µl crude enzyme extract were prepared in duplicates with volume make up with distilled water. A blank without enzyme and NBT was set to calibrate spectrophotometer. Another control having NBT but no enzyme was also set as a reference control for each treatment. All the test tubes were exposed to 400-Watt bulbs (4×100 W) for 15 minutes. The absorbance was taken at 560nm against blank immediately. Percentage of inhibition of NBT was calculated through formula given by (**Giannapolitis and Ries, 1977**).

$$\% \text{ Inhibition} = \frac{Abs\ of\ Control - Abs\ of\ Sample}{Abs\ of\ Control} \times 100$$

The 50% inhibition of reaction between riboflavin and NBT in presence of methionine is taken as 1 unit of SOD activity, the enzyme activity was expressed as unit/g FW.

f) Catalase (CAT) activity

Catalase activity was assayed in flag leaves of different wheat varieties of each replication during anthesis and 15 DAA by using procedure given by (**Chance and Maehly, 1995**). 0.2 grams of flag leaves were crushed in chilled mortar pestle using extraction buffer containing 100 mM sodium phosphate buffer having pH 6.8. The homogenate was centrifuged at 12,000g (4ºC) for 20 minutes at 4 ºC. After centrifugation, supernatant used for analyzing CAT activity. To the reaction mixture of 3ml (containing 100 Mm potassium phosphate buffer (pH 7), 0.1 µM EDTA, 20mM H_2O_2) 0.2 ml of extract was added and utilization of H_2O_2 was recorded at an interval of 5 seconds for 1 minute by measuring the decline in optical activity at 240 nm and the activity was calculated using extinction coefficient 36 M^{-1} cm-^{1} at 240 nm for H_2O_2. 1 unit of catalase activity is the amount of enzyme which breaks down 1mM of H_2O_2 per minute under assay condition.

$$\textit{CAT activity (µmole O.D } H_2O_2 \text{ dismuted)} = \frac{O.D\ \ (Abs\ per\ minute) \times 3ml\ (volume\ of\ reaction\ mixture)}{0.3ml\ (sample\ used \times (0.036)\ extenction\ cofficient}$$

$$\text{For specific enzyme activity } (\ \mu\ M/\ min/mg)\ \ = \frac{Number\ of\ enzyme\ units\ per\ ml}{concentarion\ of\ protein\ (mg/ml)}$$

YIELD AND YIELD ATTRIBUTES

a) Spike length and spike weight

Three randomly selected spike at maturity from each replication under both sowing conditions were taken. Their length (in cm) and weight (in grams) were measured with the help of centimeter scale and weighing balance.

b) Total number of spikes per plant and total weight of spikes per plant

Three plants from each replication under both sowing conditions were taken at maturity. The total number of spikes per plant were counted and their weight was also recorded with the help of weighing balance.

c) Number of spikelets per spike and grain number

Three spikes were randomly selected from each replication under both sowing conditions. Their spikelets per spike counted manually and average was computed as final result. Number of grains per spike were also counted by removing grains from its spikelet.

d) Thousand grain weight

Random sample of 100 grain were taken from each replicate of both sowing conditions, then weigh in grams (g) and converted into 1000 grain weight.

e) Biological yield

At maturity stage, plants from each replication were uprooted from ground level under 3m^2 area, bundled & labeled and then dried in sunlight to remove overnight dew, then weigh and recorded in tons/hectare as biological yield.

f) Economic yield

The total grain weight under $3m^2$ area from each replicated was harvested and finally expressed as in tons/hectare.

g) Harvest Index

Harvest index was calculated for each replication by using formula, and expressed in percentage (%)

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

h) Heat susceptibility index (H.S.I)

H.S.I was calculated over elevated temperature stress (late sowing) and non-stress condition (timely sowing) by using the formula as suggested by (**Fisher and Maurer 1978**). ***For calculation of Heat Susceptibility Index;***

$$HSI = \frac{\frac{[1-YD]}{YP}}{D}$$

Where,

YD = *mean of the genotypes in a stress environment.*

YP = *mean of the genotypes under a non-stress environment.*

$$D = 1 - \frac{mean\ YD\ of\ all\ genotypes}{mean\ YP\ of\ all\ genotypes}$$

DEVELOPMENTAL STUDY

a) Days to heading

The stage of crop when 80 % shoots were showing heading, the number of days from sowing date to this stage was calculated and expressed as number of days taken to heading.

b) Days to anthesis

When 80% of emerged ear heads exhibited the appearance of anthesis, that date was recorded. The days taken to anthesis was calculated by counting the number of days from date of sowing to 80 % date of 80 % anthesis.

c) Days taken to physiological maturity/ total life span

When 80% spikes turn yellow and enough hard the date was recorded and computation of days to physiological maturity. The number of days was taken to physiological maturity was recorded by counting the number of days from date of sowing to date of 80 % physiological maturity.

SEED QUALITY

a) Total carbohydrate content

Carbohydrate content in seeds of different wheat varieties was estimated by using method given by **Hedge *et al*., 1962.**

Reagents

- **2.5N HCl**
- **Anthrone Reagent:** 200 mg anthrone in 100 ml of ice cold 95% H_2SO_4
- **Standard glucose stock:** Dissolve 100 mg glucose in 100 ml distilled water.
- **Working standard of glucose**: 10ml of stock diluted to 100 ml with distilled water.

In a test tube 100 mg powdered wheat seeds samples were taken with 5 ml of 2.5N HCL. Hydrolysis was done by treating the test tubes in a boiling water bath for 3 hours. After 3 hours, test tubes were cooled at room temperature. Neutralization of each sample was done by adding solid sodium carbonate until effervescence ceases. Final volume makes up to 100 ml and centrifuged. The supernatant was collected and 0.5 ml of aliquot used for analysis. Final volume makes up to 1 ml with the help of distilled water. Then 4ml of anthrone reagent (freshly prepared) were added in each sample test tube and heated for 8 minutes in boiling water bath. After that samples were rapidly cooled and OD was taken at 630 nm against blank in U.V spectrophotometer. For standard 0, 0.2, 0.4, 0.6, 0.8, and 1 ml were taken from working glucose stock solution and volume make up to 1ml with distilled water and rest of the procedure was same as followed for samples. For blank ‘0’concentarion of glucose is used.

From standard graph, by plotting concentrations of standard on X-axis verses absorbance on Y-axis, amount of carbohydrate in sample tube calculated. For 100 mg sample, the amount of carbohydrate was calculated by using formula below and finally total carbohydrate content was expressed in µg/g seed sample.

$$\text{Amount of carbohydrate present in 100 mg of sample} = \frac{mg\ of\ glucose}{Volume\ of\ test\ sample} \times 100$$

b) Total starch content

Starch content in seeds of different wheat varieties was estimated by using method given by **Hedge *et al.*, 1962.**

Reagents

- **Anthrone Reagent:** 200mg anthrone in 100ml of ice cold 95% H_2SO_4
- **80% Ethanol**
- **52% Perchloric acid**
- **Standard glucose stock:** Dissolve 100mg glucose in 100ml distilled water.
- **Working standard of glucose**: 10ml of stock diluted to 100ml with distilled water.

In a test tube 100 mg powdered wheat seeds samples were taken with hot 80% ethanol to remove sugars. Centrifuged and retained the residue. Washing of residue done repeatedly with hot 80% ethanol till the washing do not give color with anthrone reagent. Then residue was dried over water bath. To the residue 5 ml of distilled water and 6.5 ml of 52% perchloric acid added. After a short 0 ºC chilling treatment, the residues were centrifuged and supernatant was saved. Same step of extraction with 52% perchloric acid repeated twice and finally volume makes up to 100 ml. 0.5 ml of aliquot used for analysis. Final volume makes up to 1 ml with the help of distilled water. Then 4ml of anthrone reagent (freshly prepared) were added in each sample test tube and heated for 8 minutes in boiling water bath. After that samples were rapidly cooled and OD was taken at 630 nm against blank in U.V spectrophotometer. For standard 0, 0.2, 0.4, 0.6, 0.8, and 1ml were taken from working glucose stock solution and volume make up to 1ml with distilled water and rest of the procedure was same as followed for samples. For blank ‘0’concentarion of glucose is used.

From standard graph, by plotting concentrations of standard on X-axis verses absorbance on Y-axis, amount of glucose content in sample tube calculated and to obtain starch content, obtained value multiplied by a factor is 0.9 and expressed as µg per gram seed sample.

c) Amylose content

Total amylose content in seeds of different varieties of wheat was estimated by using method given by **McCready and Owens, 1950.**

Reagents

- **NaOH (1 N)**
- **Phenopthaline (1 %)**
- **HCl (0.1N)**
- **Distilled Ethanol**
- **Iodine Reagent -** 200 mg of Iodine & 2 g of potassium iodide (KI) dissolved in distilled water and volume make up to 100 ml.
- **Amylose -** for standard, 100 mg amylose was taken and dissolved in 10 ml of 1 N NaOH & final volume make up to 100 ml with distilled water.

In a clean test tube, 100 mg of powdered seed sample from each variety with replication were taken and 1 ml of distilled ethanol were added and mixed well and 10 ml of 1 N NaOH further added to it. Leave this mixture for atleast 12 hours and after that final volume made up to 100 ml with distilled water and used for estimation of amylose. 2.5 ml from each diluted sample were taken in clean test tube and 20 ml of distilled water added in each test tube followed by 3 drops of phenopthaline and then drop wise 0.1N HCl added till color disappeared. Then 1 ml of iodine reagent was added in each test tube and final volume made up to 50 ml with distilled water. After that OD was taken by U.V visible spectrophotometer at 590 nm against blank (1 ml iodine reagent was taken with 49 ml of distilled water. For standard, 0.2, 0.4, 0.6, 0.8

and 1ml from standard stock were drawn into different dry and clean test tubes and 20 ml of distilled water added in each test tube followed by 3 drops of phenopthaline and then drop wise 0.1N HCl added till color disappeared. Then 1 ml of iodine reagent was added in each flask and final volume made up to 50 ml with distilled water at last OD was taken at 590 nm. Amylose was calculated by using standard graph and expressed in µg/gram seed sample.

Note: The amount of amylopectin (µg/g seed sample) is obtained by subtracting the amylose content (µg/g seed sample) from total starch content (µg/g seed sample).

d) Grain nitrogen content

Reagents and requirements

- **Catalyst mixture -** 1g selenium + 1g copper sulphate + 20 g potassium sulphate
- **Sodium Hydroxide (2.5%) -** 2.5 g NaOH was dissolved in distilled water and made up to 1 liter in a volumetric flask, mixed well and stored in plastic container.
- **Standard Sulphuric acid** (0.02N)
- **Mixed indicator -** 0.07 g methyl red was dissolved with 0.1g of bromo - cresol green in 100ml of ethanol (95%).
- **Boric acid indicator -** 20 g of pure boric acid (H_3BO_3) dissolved in 700 ml of hot distilled water and transferred to 1liter volumetric flask that contained 200 ml of ethanol and 20 ml of mixed indicator and cooled. Then 0.05 N NaOH added to the solution until reddish purple color appeared. Dilute the solution with distilled water up to 1 liter.

Grain nitrogen content in seeds of different varieties of wheat was estimated by using methodology given by (**Kjeldahl, 1883) and (Subbiah and Asij, 1956).** In a conical flask**,** 100mg seed sample along with 2g of catalyst mixture and 5ml of H_2SO_4 was digested on hot plate for 20 to 40 minutes or till the solution become colorless and odorless. Then this digested seed samples were directly transferred to steam distillation flask of Gerhardt micro-kjeldahl unit along with 5 ml distilled water. 2.5 % NaOH was also added to the solution and run for steam distillation (10 minutes). Liberated ammonia was collected in an Erlen meyer flask (250 ml) containing 20 ml of boric acid solution with mixed indicator. With the absorption of ammonia pink color of boric acid turned to green. Then final boric acid solution with ammonia taken in separate conical flask and titrated against 0.02 N H_2SO_4 with the help of burette and end point of conversion of color from green to again pink was recorded. Blank was also run by taking distilled water in the place of soil sample Nitrogen availability was calculated by putting this titrated reading in formula,

$$\text{Available nitrogen (N\%)} = \frac{[(\text{Sample} - \text{Blank}) \times 0.04 \times 14.01}{100\ (wt.of\ sample\ in\ mg)} \times 100$$

e) Total soluble protein and storage protein

Extraction of total soluble protein

200 mg wheat flour from each replicate under both sowing conditions were grounded with 3ml of cold extraction buffer of pH 7.2 containing EDTA (5 Mm), NaCl (50Mm) and Na phosphate (25mM) in it. During grinding in mortar and pestle, a final concentration of 2 mM PMSF (phenyl methyl sulphonyl fluoride) from 0.1 M stock solution was also added. The mixture was then centrifuged at 10,000g for 20 minutes at 4 ºC. The supernatant was collected in aliquots and stored at -20 ºC.

Extraction of seed storage protein

Seed storage protein in from selected wheat varieties under both sowing condition with each replication was extracted by methodology given by (**Barak *et al.*, 2015)**. For albumin, 200 mg wheat flour was suspended in 3ml of distilled water and incubated at 50ºC for 30 minutes in water bath with intermittent mixing and centrifuged at 4000g for 20 minutes at 4º C. The suspension and incubation process repeated three times after which **albumin** was retained in the soluble fraction. For globulin, 200 mg wheat flour was suspended in 3ml of 10% NaCl, mixed and centrifuged at 4000 g for 20 minutes at 4º C after which **globulin** was retained in soluble fraction. For gliadian, 200 mg wheat flour was suspended in 3ml of 75% ethanol, mixed and centrifuged at 4000g for 20 minutes at 4º C after which **gliadin** was retained in soluble fraction. For gluten, 200 mg wheat flour was suspended in 3ml of 0.2% NaOH, mixed and centrifuged at 4000 g for 20 minutes at 4° C after which **gluten** was retained in soluble fraction.

Protein content

Protein content in seeds of different wheat varieties was estimated by using method given by **Bradford (1976)**.

Reagents

- **Coomassie Brilliant Blue G-250 (CBBG-250) dye -** 50 mg CBBG-250 dye dissolved in 25 ml of ethanol and 50 ml of orthophosphoric acid (85%) w/v added, then the volume makes up to 500 ml with distilled water and solution was filtered and kept at 4ºC.
- **Bovine Serume Albumine (BSA) -** For standard, BSA is used (1mg in 1ml stock) prepared by dissolving 25 mg BSA in 25 ml of distilled water.
- **Extraction Buffer (Phosphate buffer)** – 0.3M, potassium phosphate buffer of pH 7.2

40µl of supernatant from each protein sample were taken in clean test tube and volume made up to 1 ml by extraction buffer then 3 ml of dye was added to all test tube and incubated in dark till development of blue color and OD was taken at 595 nm and the amount of protein present in the sample was calculated from BSA standard curve. For standard curve, 10, 20, 30, 40, 50, 60, 70, 80. 90 µl from standard stock were drawn into different dry and clean test tubes. The volume was made up to 300 µl by adding extraction buffer/ distilled water. Then 3 ml of dye was added in each test tube and incubated in dark till development of blue color and OD was taken at 595 nm. For blank distilled water was used in place of BSA.

e) Analysis of protein sample by SDS Polyacrylamide gel electrophoresis

To find out the changes in total protein and storage protein profiles associated with different wheat varieties under different sowing conditions (D1 and D2) protein analysis was done by one dimensional Sodium dodesyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) using methodology described by (**Laemmli, 1970**).

Principle

Sodium dodesyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) is the most widely used method for analyzing proteins qualitatively. SDS is an anionic detergent which provides the same negative charge to all proteins. The method is based on the separation of protein peptides according to size under forced electric field. A polyacrylamide gel with acrylamide content above a critical density restrains larger molecules from migration as fast as smaller molecules. As SDS provides negative charge to all protein samples so final separation of protein peptides is purely depends on their difference in molecular weight.

Standard Solutions:

- **30% Acrylamide and bis- acrylamide stock –** 14.6 g of acrylamide and 0.49 g of N, N'- methylene bis acrylamide dissolved in 50ml autoclaved double distilled (ddw) water. Warmed slightly for dissolving it completely. Filtered and stored at 4ºC in a dark-colored bottle.
- **10% SDS –** 10g SDS was dissolved in 100ml autoclaved double distilled water Kept at 37ºC or warmed slightly for complete dissolution.
- **Stacking gel buffer** - 0.5M Tris-HCL (pH- 6.8).
- **Separating gel buffer** -1.5M Tris-HCL (pH- 8.8).
- **Electrophoresis gel buffer- Tris -glycine buffer (**pH- 8.3)

Tris base	25mM
Glycine	192mM
SDS	0.1%

- **Sample buffer (2x) –** It was prepared 10 ml and distributed in 1ml each in eppendorf tube and kept in freezer. As it kept separated so we have to add β-mercapto ethanol (BME) freshly before use.

Tris HCL (pH- 6.8)	62mM
SDS	2%
Glycerol	10%
BME (β- mercapto ethanol)	5%
EDTA (ethylenediamine tetra acetic acid)	0.1%
BPB (Bromo phenol blue)	0.1%

- **Fixing gel –** 50% methanol and 10% glacial acetic acid
- **Staining solution** - 50% methanol, 10% glacial acetic acid and 0.1% CBB-R 250 Dye
- **Destaining solution-** 10% methanol and 7% glacial acetic acid
- **10% Ammonium per sulpahte** – 0.1g/ml

Procedure

1. **Composition of 10% separating gel (20ml)**

30% Acrylamide stock	8.0ml
Separating gel buffer	5.0ml
10% SDS	0.1ml
10% APS	0.2ml
TEMED	10µl
DDW	6.69ml

2. **Composition of 5% stacking gel (8ml)**

30% Acrylamide stock	1.3ml
Stacking gel buffer	2.0ml
10% SDS	80µl ml
10% APS	80µl ml
TEMED	10µl
DDW	4.53ml

Note: *APS and TEMED was just added before poring of gel solution as TEMED initiates the cross-linking of gel.*

3. The gel plates were assembled with spacers and clamps and the bottom was sealed with 1 % agarose. The 10% separating gel was poured up to 3/4th height of the plates and over laid with butanol. It was allowed to polymerize at 37ºC for 40 minutes.
4. Once the polymerization was done a distinct interface sets between gel and butanol. At this point, the over laid butanol was removed and 5% stacking gel was poured and comb inserted.
5. The stacking gel was left to polymerize for about half an hour. After polymerization the comb was removed and wells were cleaned with a syringe.
6. The gel plates were assembled in electrophoresis chamber filled with electrophoresis.
7. **Sample preparation -** Approx. 200µg of protein sample taken with 2x sample (100µl) buffer in 2:1 ratio. This was boiled in a boiling water bath for 5 minutes. Then the samples were centrifuged at 10,000g for 10 minutes. A medium ranged protein marker (14. 30KDa – 97.40KDa) was also run with the samples.
8. 20µl from each sample was loaded into the wells with the help of micropipette with special loading tips. The gel was run at 80 volts till the sample was stacked after that the voltage was raised to 100 volts. The gel was allowed to run till the dye reaches 0.5cm from the lower edge of the gel.
9. After completion of electrophoresis, gel was taken out in a tray containing the fixing solution and the gel was left for 30 minutes in fixing solution.
10. Then after 30 minutes the gel transferred to staining solution for 4 hours.
11. After 4 hours, the gel was taken out and kept in detaining solution and left overnight.
12. Next day detained gel with clear bands achieved.

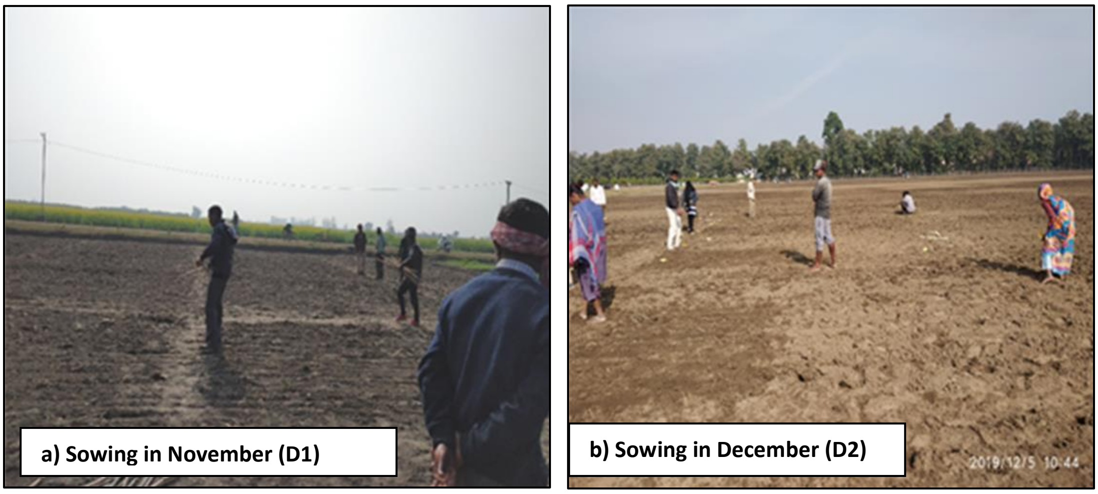
3.9 STATISTICAL ANALYSIS

The statistical analysis was done with software STPR 15 and STPR 2 obtained from department of Mathematics, statistics and Computer, College of basic sciences and Humanities G.B Pant University of Agriculture and Technology.

3.10 CORRELATION ANALYSIS

Correlation coefficient were worked out between parameters with the help of SPSS software version 26.

FIELD VIEW OF EXPERIMENTAL SITE


a) Sowing in November (D1)

b) Sowing in December (D2)

Figure 3.1: Field view at the time of sowing under both sowing conditions.


a) Sowing in November (D1)

b) Sowing in December (D2)

Figure 3.2: Field view at the time of vegetative stage under both sowing conditions

a) Sowing in November (D1)

b) Sowing in December (D2)

Figure 3.3: Field view at the time of anthesis under both sowing conditions

a) Sowing in November (D1)

b) Sowing in December (D2)

Figure 3.4: Field view at the time of maturity under both sowing conditions

Figure 3.5: Sample collection for data analysis

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

The present investigation was conducted to study the effect of elevated temperatures on various growth and development related parameters of eight wheat varieties namely; **UP2628, HD3086, UP2967, UP2784 (**recommended for timely sowing) **UP2526, UP2565, UP2748 and HD3059 (**recommended for late sowing) during two consecutive years 2018-19 and 2019-20 in the months of November (D1) and December (D2). Temperature variation due to D2 was observed for various growth stages of wheat life cycle. According to meteorological data of both the years 2018-19 and 2019-20, an increment of (2.85 ºC/4.65 ºC) in average maximum to minimum temperature during both years at tillering, (3.25 ºC /0.55 ºC), at heading, (5.22 ºC /2.29 ºC), at anthesis and (3.46 ºC /2.37 ºC) at grain filling (15 DAA) was reported under D2 as compared to D1. On the other hand, an elevation of (3.75ºC) at the time of tillering, (1.89 ºC) at heading, (3.75 ºC) at anthesis and (2.91 ºC) at the time of grain filling was reported on average temperature of two years trial 2018-19 and 2019-20 under D2 as compared to D1. The results based on statistically analysed data pertaining to the experiments performed during the course of investigation along with relevant discussion are presented below.

4.1 Effect of elevated temperatures on morphological characteristics

4.1.1 Plant height

Plant height of different wheat varieties was recorded at maturity in two consecutive years 2018-19 and 2019-20. Plant height was reduced due to D2 (elevated temperatures) in both years at maturity. During 1st trial (2018-19), maximum height was recorded in HD3086(95.11cm) and minimum in UP2748 (82.11 cm) under D1. While, under D2, plant height was found to be reduced and maximum height was recorded in UP2967 (88.67cm) and minimum in UP2748 (77.33 cm). Similarly, during 2nd trial (2019-20), highest plant height was observed in HD3086 (94.66 cm) and lowest in UP2748 (80.33 cm) under D1. While under D2, highest height was observed UP2967 (87.89 cm) and lowest in UP2748 (72.44 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that at maturity maximum plant height was achieved by HD3086 variety (94.89 cm) and minimum by UP2748 (81.22 cm) under D1. However, under D2 maximum height was recorded for UP2967 (88.28 cm) and minimum in UP2748 (74.89 cm) (**Table 4.1.1**). Over all at the time of maturity maximum reduction in plant height due to D2 (elevated temperatures) was observed in UP2628 (16.27 %) while minimum reduction in plant height was observed in UP2565 (1.69 %) and UP2526 (1.71%) (**Figure 4.1.1**). Statistically, a significant difference was observed in values of plant height at the time of maturity between the two-sowing conditions (D1 and D2) between varieties as well as between years ($p \leq 0.01$). All the interactions were also found significant with each other ($p \leq 0.01$) except the interaction between year × treatment × varieties that was found non-significant ($p > 0.05$).

Table 4.1.1 Effect of differential temperatures on plant height (cm) of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

Plant height (cm)						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	92.50	92.66	**92.58**	78.33	76.74	**77.54**
HD3086	95.11	94.66	**94.89**	81.33	80.33	**80.83**
UP2967	93.56	94.11	**93.83**	88.67	87.89	**88.28**
UP2784	93.17	94.33	**93.75**	86.44	84.33	**85.39**
UP2526	85.89	85.76	**85.82**	85.33	83.39	**84.36**
UP2565	84.32	85.67	**84.99**	83.16	83.94	**83.55**
UP2748	82.11	80.33	**81.22**	77.33	72.44	**74.89**
HD3059	85.33	86.11	**85.72**	82.00	83.56	**82.78**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.134		0.269		0.381	
CD (5%)	0.381		0.763		1.07	

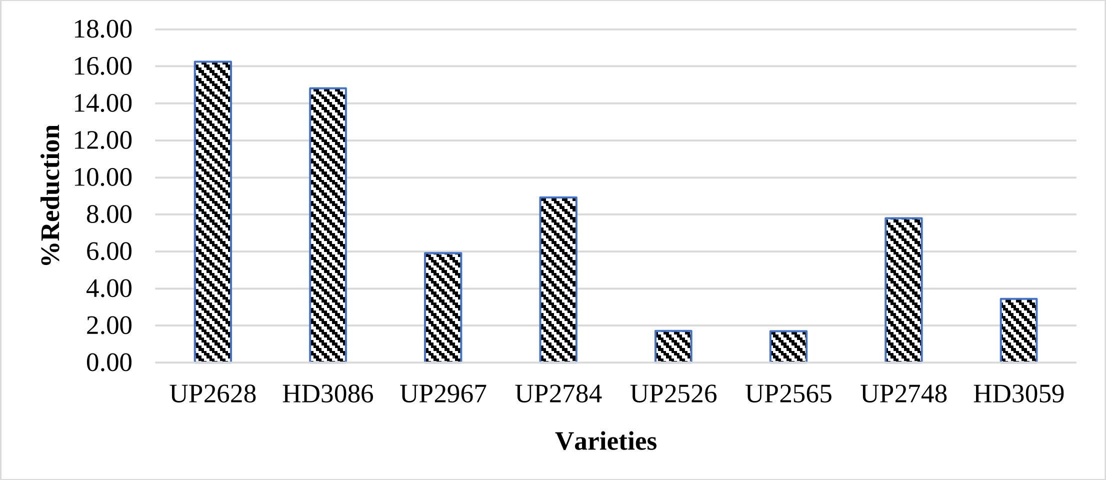


Figure 4.1.1 Reduction in mean values of plant height of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present research plant height was found decreased in all wheat varieties under D2 condition as compared to D1. Previous researches also showed that D2 schedule negatively affects shoot length of plant. As elevated temperatures leads to alter various physiological functions such as cellular and photosynthetic activity in various wheat crop species and hence reduces shoot length (**Stones and Nicolas, 1995**). Reduction in plant height is mainly due to forced senescence when plant exposes to elevated temperatures (heat stress) due to inappropriate sowing timings. As heat stress during anthesis which affects over all plant height at maturity (**Kanchan and Mahendra, 2000**).In an experiment, plant height was found declined by28% due to heat stress and had a negative correlation with grain yield which is responsible for poor yielding of wheat cultivars (**Modarresi *et al*., 2010**).Similarly, a reduction of 13.85% in PAK-81, 8.70% in LU-26S, wheat varieties was observed in plant height due to elevated temperature when they were under delayed sowing conditions (**Ali *et al*., 2016**). An experiment with different wheat varieties under different sowing conditions also concludes that the reduction in plant height under elevated temperatures is due to fast phasic change and due to which vegetative phase become short and reproductive come early. During investigation plant height was found reduced upto 30.75% in HD 2733 and 24.15% in K9006 wheat varieties under the heat stress condition. (**Tiwari *et al*., 2017**). Similarly, a reduction up to8% in plant height was observed in various wheat genotypes, such as SH-Salt-6, NRL-1236, NIA-Sarang, LU-26s and Dani that were found failed to cope up elevated temperature and showed reduced plant height under elevated temperatures (**Khan *et al*., 2020).**

4.1.2 Tiller number and Productive tillers

At anthesis, number of tillers and productive tillers were analysed in different wheat varieties. The results showed a reduction in number of tillers and productive tillers under D2 (elevated temperatures) condition as compared to D1. During 1st trial (2018-19), maximum number of tillers was observed in UP2784 variety (6.55) and minimum in UP2526 (5.46) under D1. While under D2, tiller number was reduced and maximum value was observed in UP2565 (5.49) and minimum in UP2784 variety (5.00). Similarly, during 2nd trial (2019-20), highest tiller number was observed in UP2784 (6.58) and lowest inUP2748 (5.00) under D1 sowing conditions. While under D2, highest number of tillers was observed in UP2565 (5.67) and lowest in UP2967 (4.50). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum tiller number was achieved by UP2628 variety (6.20) and minimum by UP2748 (5.20) under D1. However, under D2maximum tillers was recorded for UP2565 (5.58) and minimum in UP2967 (4.53) (**Table 4.1.2**). Over all maximum reduction in tiller number due to D2 was observed in UP2784 (22.98 %) while minimum reduction in number of tillers was observed in UP2526 (2.59%) and UP2565 (2.14%) (**Figure 4.1.2**). Statistically, a significant difference in tiller number was observed during both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was observed between the two years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant ($p \leq 0.01$).

Productive tillers were also found to be reduced under D2 (elevated temperatures) in comparison to D1. During 1st trial (2018-19), maximum productive tillers were observed in HD3086 variety (5.83) and minimum in UP2748 (4.67) under D1. Under D2 sowing conditions, productive tillers were reduced and maximum value was found in UP2565 (5.11) while minimum in UP2748 variety (4.11). During 2nd trial (2019-20), highest productive tillers observed in UP2628 (5.80) and lowest in UP2748 (4.17) under D1 condition while under D2, highest productive tillers were observed in UP2565 (5.56) and lowest in UP2784 (3.44). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum productive tiller was recorded in HD3086 variety (5.67) and minimum in UP2748 (4.42) under D1. However, under D2 maximum productive tiller was observed for UP2565 (5.33) and minimum for UP2748 (4.04) (**Table 4.1.2**). Over all maximum reduction in number of productive tillers due toD2(elevated temperatures) was observed in UP2784 (27.32%) while minimum in UP2565 (2.07%) (**Figure 4.1.2**). Statistically, there was a significant difference in values of productive tillers for both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was observed between the two years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant ($p \leq 0.01$).

Table 4.1.2 Effect of differential temperatures on number of tillers and productive tillers of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

Tiller number							**Productive tillers**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	6.17	6.22	**6.20**	5.33	5.07	**5.20**	5.33	5.80	**5.56**	4.67	4.89	**4.78**
HD3086	6.00	6.17	**6.08**	5.07	5.66	**5.37**	5.83	5.50	**5.67**	4.92	5.00	**4.96**
UP2967	6.00	5.75	**5.88**	4.56	4.50	**4.53**	5.33	5.00	**5.17**	4.50	4.39	**4.44**
UP2784	6.55	6.58	**6.56**	5.00	5.11	**5.06**	5.00	5.70	**5.35**	4.33	3.44	**3.89**
UP2526	5.46	5.44	**5.45**	5.37	5.25	**5.31**	5.33	5.19	**5.26**	5.07	5.00	**5.04**
UP2565	5.52	5.89	**5.70**	5.49	5.67	**5.58**	5.22	5.67	**5.45**	5.11	5.56	**5.33**
UP2748	5.50	5.00	**5.25**	5.11	4.59	**4.85**	4.67	4.17	**4.42**	4.11	3.96	**4.04**
HD3059	5.67	5.70	**5.68**	5.33	5.22	**5.27**	5.33	5.44	**5.38**	5.00	5.20	**5.10**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.057		0.115		0.163		0.072		0.145		0.205	
CD (5%)	0.163		0.327		0.463		0.204		0.409		0.579	

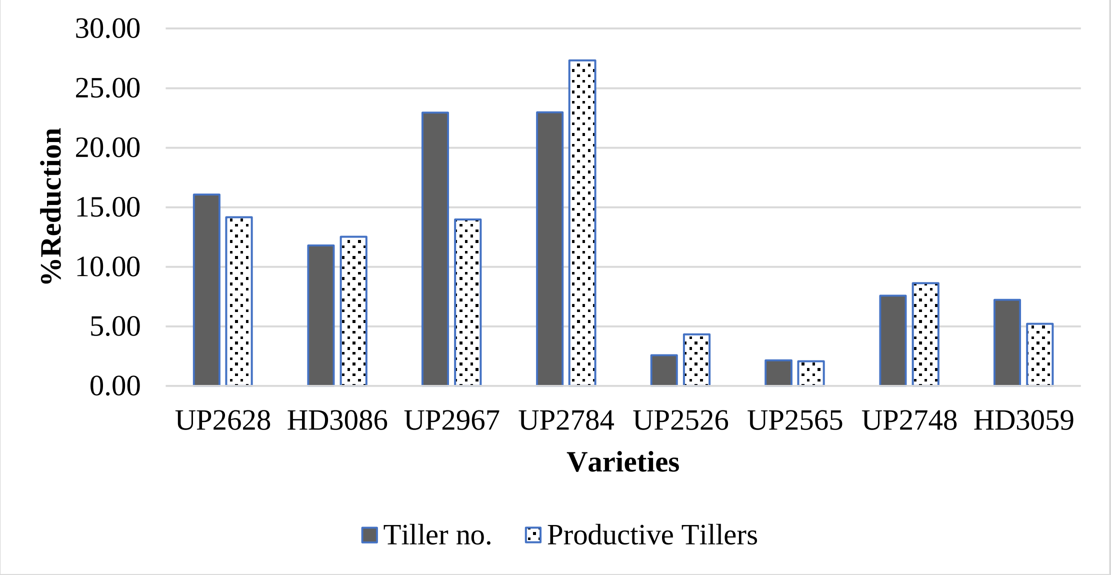


Figure 4.1.2 Reduction in mean values of tiller number and productive tillers of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

The value of tiller number and productive tillers are found to be reduced under D2 as compared to D1 conditions in all wheat varieties. These parameters can be considered as a good indicator for identifying the yield potential of the wheat crop. Decrement in such parameter considered harmful to its potential of producing good yield under stress condition (heat stress). Similar trend of reduction in tiller number and productive tillers under thermal stress condition showed by previous research in which wheat cultivars namely; HD2285, P10066, Sonalika, P10423 and WH291 were sown on three different months (25 Dec., 4 Jan. and 15 Jan.). The results showed that as with later sowing dates tiller number and effective tillers per plant were found reduced in comparison to early sowing dates (**Bisnoi and Taneja, 1990).** Due to delayed sowing plant experiences heat stress during its growth period which results improper growth and development of tillers and ultimately productive tillers (**Nelson, 1988**). Forced maturity due to elevated temperatures also one of the reasons of improper tiller development in wheat crop (**Kanchan and Mahendra, 2000).** The general trend of reduction in number of shoots and ear bearing shoots in wheat as a result of elevation in temperature has also been reported by (**Mishra, 2002)**. Through an experiment**,** it is reported that upto15.38% reduction in tiller number was observed under heat stress conditions indifferent wheat varieties in comparison to non-heat stress conditions (**Riaz-ud-Di *et al.*, 2010).** A reduction of 22% in tiller number and 31.90% in grain yield was observed in wheat under elevated temperature as compared to normal conditions (**Sharma *et al.*, 2014).** Late plantation (elevation in temperature) in some wheat varieties causes reduction upto68.53% in the number of tillers per plant especially in K 9006 and Halna variety of wheat (**Tiwari *et al.*, 2017**). Previously, the maximum reduction was recorded in wheat yield (19.12%) followed by number of tillers (17.6%) indicates that these traits are highly influenced by environmental factors i.e heat stress (**Kumar *et al.*, 2018**). Similar trend also followed by a research in which the number of tillers per plant along with percentage of tiller emergence and number of productive tillers per plant was significantly decreased by the delay in sowing pattern or inappropriate sowing in different wheat varieties. By some recent study in which about 30% reduction in tillers per meter square was observed under late sowing of wheat when 20 wheat varieties were analysed for two consecutive years under different sowing schedule (**Kumar *et al*. 2020; Toyota and Morokuma, 2021)**.

4.1.3 Flag leaf length and width

At anthesis, top most leaf (flag leaf) of all wheat varieties was analysed in terms of its length, width and area under both sowing conditions. The results showed reduction in overall morphological structure (length width and area) of flag leaf under D2 in comparison to D1. During 1st trial (2018-19), the flag leaf length was found maximum for UP2748 variety (23.63 cm) and minimum for HD3059 (18.11 cm) under D1 while under D2, the length of flag leaf was reduced and found maximum for HD3086 (21.59 cm) and minimum for UP2628 variety (15.67 cm). Similarly, during 2nd trial (2019-20), highest length recorded in UP2748 (23.00 cm) and lowest in UP2628 (18.44 cm) under D1 while under D2 the length was reduced & highest length observed was in UP2748 (21.67 cm) and lowest in UP2628 (15.89 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum length of flag leaf was achieved by UP2748 variety (23.32 cm) and minimum by HD3059 (18.65 cm) under D1. However, under D2 maximum length was observed for UP2748 (21.22 cm) and minimum for UP2628 (15.78)

(**Table 4.1.3**). Over all maximum reduction in length of flag leaf due to D2 (elevated temperatures) was observed in UP2565 (18.70 %) while minimum reduction in HD3086 (3.83 %) (**Figure 4.1.3**). Statistically data revealed that there is a significant difference in values of length of flag leaf between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was observed between the two years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant ($p \leq 0.01$).

For the width of flag leaf, during 1st trial (2018-19) maximum flag leaf width was observed for HD3086 variety (1.83 cm) and minimum for UP2628 variety (1.31 cm) under D1 while under D2, the width of flag leaves was reduced and the maximum value observed in UP2628 (1.30 cm) & minimum in UP2784 (0.89 cm).Similarly, during 2nd trial (2019-20), highest width of flag leaf was observed in HD3086 (1.94 cm) while lowest width observed in three wheat varieties (UP2628, UP2784 and HD3059 (1.41 cm) under D1 while under D2, the width was reduced &maximum width observed in UP2628 (1.32 cm) and minimum in UP2784 (0.87cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that highest value of width of flag leaf was achieved by HD3086 variety (1.89) and lowest by UP2628 (1.14 cm) under D1. However, under D2 maximum value of flag leaf width observed for UP2628(1.31 cm) and minimum for UP2784 (0.88 cm) (**Table 4.1.3**). Over all maximum reduction in width of flag leaf due to D2 (elevated temperatures) was observed in HD3086 (44.22 %) while minimum reduction inUP2628 (3.68%) (**Figure 4.1.3**). Statistically there was a significant difference in values of width of flag leaf between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was observed between the two years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant ($p \leq 0.01$).

Table 4.1.3 Effect of differential temperatures on flag leaf length and width of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

	Flag leaf length (cm)						**Flag leaf width (cm)**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	19.00	18.44	**18.72**	15.67	15.89	**15.78**	1.31	1.41	**1.14**	1.30	1.32	**1.31**
HD3086	21.63	22.00	**21.82**	21.59	20.37	**20.98**	1.83	1.94	**1.89**	1.05	1.06	**1.05**
UP2967	20.22	22.13	**21.18**	17.26	17.96	**17.61**	1.42	1.48	**1.45**	1.09	1.07	**1.08**
UP2784	22.63	22.93	**22.78**	19.3	19.5	**19.40**	1.49	1.41	**1.45**	0.89	0.87	**0.88**
UP2526	21.48	20.45	**20.96**	19.22	19.48	**19.35**	1.75	1.63	**1.69**	1.02	1.05	**1.04**
UP2565	21.11	22.56	**21.83**	17.83	17.67	**17.75**	1.65	1.64	**1.64**	1.10	1.15	**1.13**
UP2748	23.63	23.00	**23.32**	20.78	21.67	**21.22**	1.72	1.71	**1.71**	1.08	1.05	**1.06**
HD3059	18.11	19.18	**18.65**	17.48	17.17	**17.32**	1.49	1.41	**1.45**	1.06	1.03	**1.04**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.138		0.276		0.391		0.014		0.029		0.041	
CD (5%)	0.391		0.782		1.107		0.041		0.082		0.116	

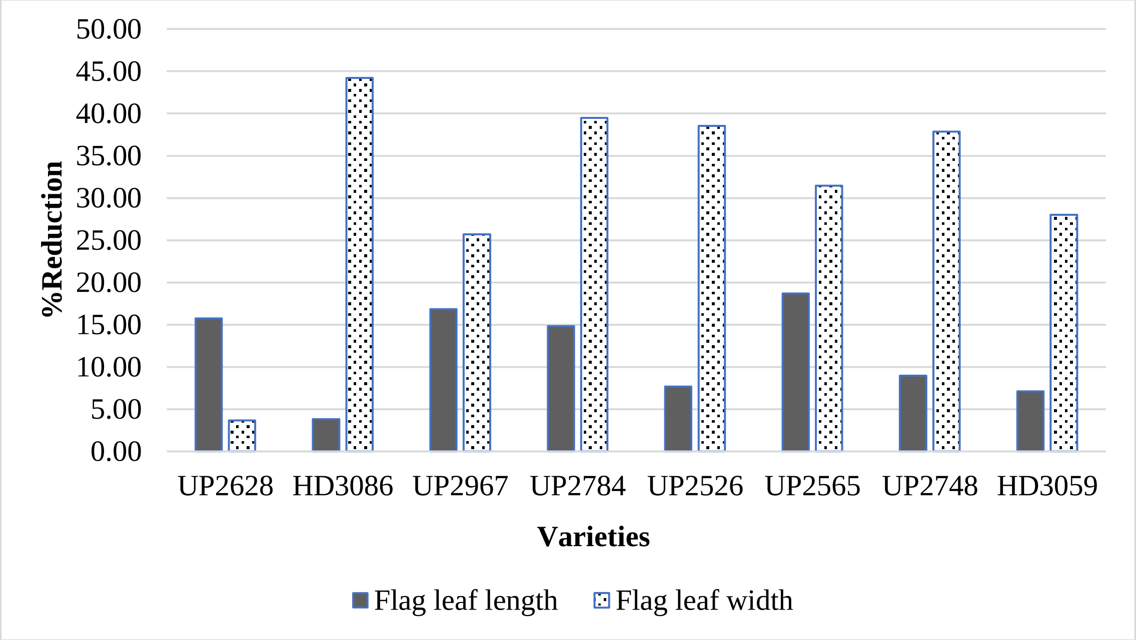


Figure 4.1.3 Reduction in mean values of flag leaf length and width of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.1.4 Flag leaf Area

As the leaf area is concerned, during 1st trial (2018-19) maximum area of flag leaf was observed for UP2748 variety (28.56 cm^2) and minimum for UP2628 variety (18.52 cm^2) under D1 while under D2, the flag leaf area was found to be reduced and the maximum flag leaf area was observed in UP2748 (17.79 cm^2) & minimum in UP2784 variety (12.85 cm^2). Similarly, during 2nd trial (2019-20), highest area of flag leaf was observed for UP2565 variety (27.71 cm) while lowest for UP2628 (18.21 cm^2) under D1 while under D2, the area was reduced & highest area was observed inUP2748 (17.06 cm^2) and lowest in UP2784 variety (12.73 cm^2).On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that highest value of leaf area of flag leaf was achieved by UP2748 variety (27.96 cm^2) and lowest by UP2628 (18.37 cm^2) under D1. However, under D2maximum value of flag leaf area was observed for UP2748 (17.42 cm^2) and minimum for UP2784 (12.79 cm^2) (**Table 4.1.4**). Over all maximum reduction in flag leaf area due to D2was observed in UP2784 (48.36 %) while minimum reduction was observed in UP2628 (26.78%) (**Figure 4.1.4**). Statistically, there was a significant difference in values of area of flag leaf between both sowing conditions (D1 and D2) and between varieties ($p \le 0.01$) while a non-significant difference was observed between the two years data ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant ($p \le 0.01$).

Table 4.1.4 Effect of differential temperatures on flag leaf area (cm^2) of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

Flag leaf Area (cm^2)						
	D1			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	18.52	18.21	**18.37**	13.40	13.50	**13.45**
HD3086	26.50	26.03	**26.27**	16.95	16.12	**16.53**
UP2967	21.52	24.66	**23.09**	14.07	14.43	**14.25**
UP2784	25.28	24.23	**24.76**	12.85	12.73	**12.79**
UP2526	28.17	24.96	**26.56**	14.78	15.36	**15.07**
UP2565	26.09	27.71	**26.90**	16.74	16.21	**16.48**
UP2748	28.56	27.36	**27.96**	17.79	17.06	**17.42**
HD3059	20.23	20.22	**20.23**	13.91	13.21	**13.56**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.225		0.450		0.637	
CD (5%)	0.637		1.274		1.802	

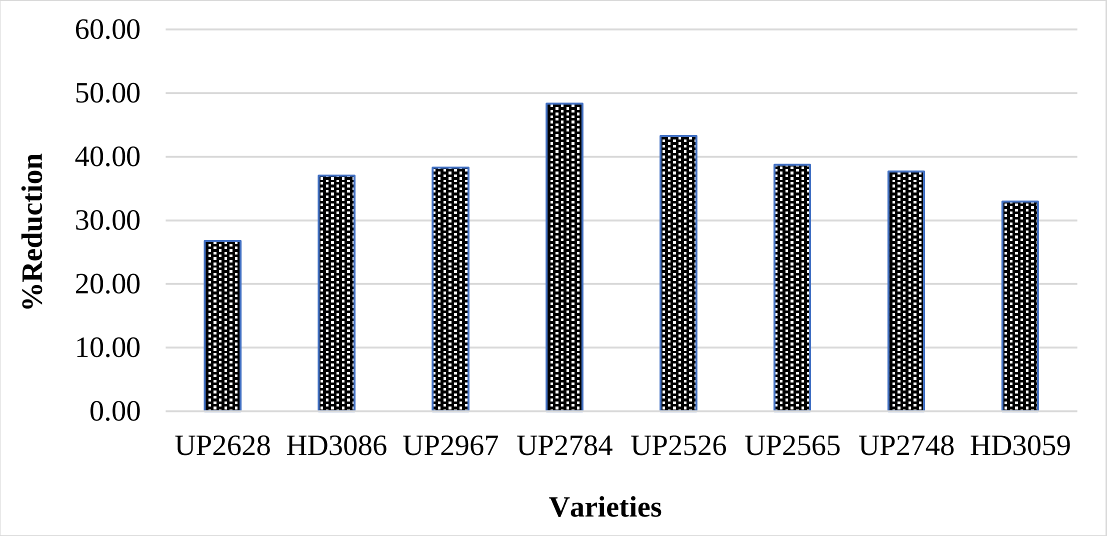


Figure 4.1.4 Reduction in mean values of flag leaf area of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

Leaf area (leaf length × width × correction Factor) is one of the important parameters that play main role in photosynthetic activity, sun light radiation use efficiency, and leaf transpiration studies in plants. It also plays a pivotal role in assimilate partitioning and stress tolerance of wheat during its terminal growth phase. Any stress condition (heat stress) lead to affects all the above physiological process and ultimately yield of crop. From previous researches in banana (*Musa acuminata* Colla.) it is clear that there is a strong relationship between leaf area and various combinations of leaf length (L), leaf width (W) and correction factor. And it is also well accepted that length and width of flag leaf analysis is one of the best and non-destructive method for leaf area estimation **(Potdar and Pawar, 1991).** In the present investigation, flag leaf length and width were found decreased due to D2 (elevated temperatures/heat stress) that ultimately decreases the flag leaf area. In previous researches also under early or normal sowing condition (non-stress), leaf area including leaf length and its width was found higher in comparison to leaf area (length and width) of flag leaf of wheat varieties which are grown delayed condition (elevated temperatures) (**Mishra, 2002).** The reason for reduced leaf area due to the changes such as reduction in cell size, partial closure of stomata, increased xylem vessels under elevated temperatures, causes negative effect on growth and development of leaf, reduced length and width that ultimately decreases overall leaf morphological structure and causes reduced leaf area (**Anonymous, 2004**).Through some experiments a high positive correlation was observed between leaf length, leaf width and leaf area which concludes that if a crop have maximum length and width of flag leaf so its leaf area is also found maximum (in case of early/normal sowing cultivars of wheat) while if a crop have less flag leaf length and width (in case of late sown wheat cultivars) showed minimum leaf area in comparison to early/normal sowing wheat cultivars. This trend also observed in which leaf area was highly correlated with the combination of lamina length (L) and lamina width (W) in *Vicia faba*L. (**Pekson 2007**). Similar trend also followed by other researches in which leaf area found to be reduced upto 40.17 % in PBW-343 wheat genotype in elevated temperatures as compared to normal sowing conditions (**Srivastava, 2005 and Srivastava, 2012)**. During an experiment, 58 genotypes of wheat were observed under two different sowing condition i.e. under stress (high temperature, sowing date - March 1) and optimal (sowing date - October 1). There was reduction in flag leaf area (21.60 %) and in flag leaf width (7.30%) was observed under stress condition as compared to optimal condition (**Youldash *et al*., 2020**). In *Poaceae* crop family, dry matter production is largely determined by the leaf area of plant which is regulated by the kinetics of emergence, growth, and senescence of tillers and leaves. According to previous reviews, it was observed that in wheat varieties under elevated temperatures when tiller number and number of leaves was reduced, as a result of it, they also negatively affect flag leaf area and dry matter accumulation and ultimately the yield of the crop (**Toyota and Morokuma, 2020).** According to recent research, leaf area of different wheat genotypes was found reduced under late sowing (due to heat stress) in comparison to normal conditions (non-stress). At normal sowing MASR-3, BWS-78, NIA-10/8, MASR-23, TJ-83, TD-1 and Kiran-95 wheat genotypes had larger leaf which was found reduced under elevated temperatures (**Laghari *et al*., 2021**).

4.1.5 Fresh weight and Dry weight of flag leaf

Fresh weight and dry weight of flag leaf was recorded for all wheat varieties under both sowing condition during anthesis. The results showed reduction in fresh and dry weight under D2 in comparison to D1. During 1st trial (2018-19) highest fresh weight of flag leaf was observed in UP2526 variety (0.596 grams) and lowest in UP2748 (0.351 grams) under D1 while under D2 the flag leaf fresh weight was reduced and the highest flag leaf fresh weight was observed in UP2526 (0.408 grams) & lowest in UP2628 variety (0.247grams). Similarly, during 2nd trial (2019-20), highest fresh weight of flag leaf was observed in UP2526 (0.547 grams) and lowest in UP2628 (0.364 grams) under D1 and under D2 highest fresh weight of flag leaf was observed again in UP2526 (0.421 grams) and lowest in UP2628 variety (0.251grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum fresh weight of flag leaf was achieved by variety UP2526 (0.572 grams) and minimum by UP2628 & UP2748 (0.359 grams for both variety) under D1 while under D2, the maximum fresh weight of flag leaf recorded for UP2526 variety (0.414 grams) and minimum for UP2628 variety (0.249 grams) (**Table 4.1.5**). Over all maximum reduction in flag leaf fresh weight due to D2 was observed in UP2967 variety (33.15 %) while minimum reduction was observed in UP2748 variety (12.41%) (**Figure 4.1.5**). Statistically, there was a significant difference in fresh weight of flag leaf was observed between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$).

For dry weight of flag leaf, during 1st trial (2018-19) highest dry weight was observed in UP2748variety (0.195 grams) and lowest in UP2628 (0.114 grams) under D1 while under D2 the flag leaf dry weight was reduced and highest dry weight observed in UP2526 (0.147 grams) & lowest in UP2565 (0.095 grams). Similarly, during 2nd trial (2019-20), highest dry weight of flag leaf was observed in UP2748 (0.194grams) and lowest in UP2628 (0.115 grams) under D1 while under D2 highest dry weight of flag leaf was observed in UP2526 (0.160 grams) and lowest in UP2628 variety (0.081grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that highest dry weight of flag leaf was achieved by variety UP2748 (0.194 grams) and lowest by UP2628 (0.115 grams) under D1 while under D2 condition, the highest dry weight of flag leaf was recorded in UP2526 variety (0.154 grams) and lowest in UP2628 variety (0.089 grams) (**Table 4.1.5**). Over all maximum reduction in flag leaf dry weight due to D2 (elevated temperatures) was observed in UP2784 variety (25.99%) while minimum in UP2526 variety (18.62%) (**Figure 4.1.5**). Statistically, there was a significant difference in dry weight of flag leaf between both sowing conditions (D1 and D2) and between varieties (p≤0.01) along with the two years data (p≤0.05). All the other interactions were also found significant with each other (p≤0.01).

Table 4.1.5 Effect of differential temperatures on flag leaf fresh weight (grams) and dry weight (grams) of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

Varieties	Fresh weight (grams)						Dry weight (grams)					
	D1			D2			D1			D2		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
UP2628	0.354	0.364	**0.359**	0.247	0.251	**0.249**	0.114	0.115	**0.115**	0.098	0.081	**0.089**
HD3086	0.412	0.420	**0.416**	0.360	0.336	**0.348**	0.123	0.137	**0.130**	0.101	0.104	**0.103**
UP2967	0.429	0.426	**0.427**	0.295	0.277	**0.286**	0.170	0.171	**0.170**	0.121	0.130	**0.126**
UP2784	0.465	0.412	**0.439**	0.345	0.338	**0.341**	0.142	0.144	**0.143**	0.107	0.105	**0.106**
UP2526	0.596	0.547	**0.572**	0.408	0.421	**0.414**	0.194	0.184	**0.189**	0.147	0.160	**0.154**
UP2565	0.401	0.450	**0.425**	0.365	0.358	**0.361**	0.128	0.121	**0.125**	0.095	0.093	**0.094**
UP2748	0.351	0.367	**0.359**	0.317	0.311	**0.314**	0.195	0.194	**0.194**	0.145	0.148	**0.147**
HD3059	0.401	0.415	**0.408**	0.326	0.327	**0.327**	0.143	0.143	**0.143**	0.109	0.107	**0.108**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.009		.018		0.026		.009		0.019		0.028	
CD (5%)	0.026		.052		0.074		.002		0.056		0.079	

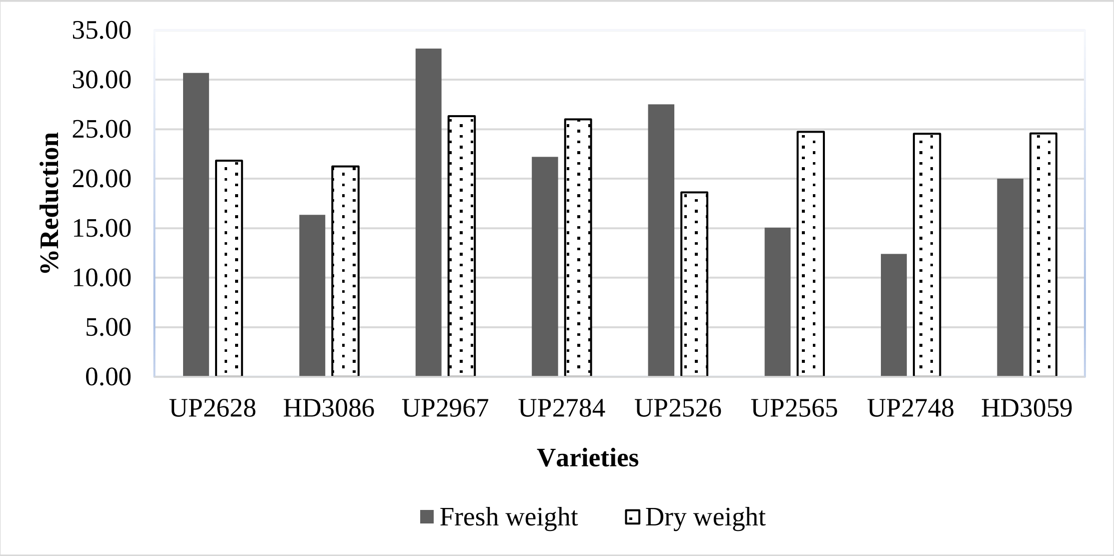


Figure 4.1.5 Reduction in mean values of flag leaf fresh weight and dry weight of different varieties under D2as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.1.6 Specific leaf weight (SLW)

Specific leaf weight (SLW) of all wheat varieties was also calculated during anthesis. The results revealed a decrement in values of specific leaf weight under D2 in comparison to D1. During 1st trial (2018-19) highest SLW was observed in UP2967 variety (8.536 mg/cm^2) and lowest in HD3086 variety (5.685 mg/cm^2) under D1 while under D2 the SLW was decreased. The highest value of SLW under D2 was observed in UP2967 variety (7.730 mg/cm^2) &lowest in HD3086 (4.608 mg/cm^2). Similarly, during 2nd trial (2019-20), highest SLW was recorded in HD3059 variety (7.734 mg/cm^2) and lowest in HD3086 (5.989 mg/cm^2) under D1 while under D2, the value of SLW was decreased and highest SLW observed in HD3059 variety (7.570 mg/cm^2) and lowest in HD3086 variety (4.219 mg/cm^2). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value of SLW was achieved by UP2967 variety (7.976 mg/cm^2) and minimum by HD3086 (5.837 mg/cm^2) under D1 while under D2, the maximum SLW was recorded inHD3059 variety (7.367 mg/cm^2) and minimum in HD3086 variety (4.413 mg/cm^2) (**Table 4.1.6**). Over all maximum reduction in SLW due to D2 (elevated temperatures) was observed in HD3086 variety (24.39 %) while minimum in UP2565 variety (0.45%) (**Figure 4.1.6**). Statistically, there was a significant difference in specific leaf weight between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there is non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$).

Table 4.1.6 Effect of differential temperatures on specific leaf weight (mg/cm^2) of flag leaf of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

Specific leaf weight (mg/cm^2)						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	6.475	6.397	**6.436**	5.297	5.363	**5.330**
HD3086	5.685	5.989	**5.837**	4.608	4.219	**4.413**
UP2967	8.536	7.416	**7.976**	7.730	6.888	**7.309**
UP2784	7.918	7.331	**7.624**	6.080	6.989	**6.535**
UP2526	7.514	7.388	**7.451**	7.373	7.112	**7.243**
UP2565	5.852	6.387	**6.120**	5.820	6.365	**6.092**
UP2748	7.790	7.589	**7.689**	7.100	7.357	**7.228**
HD3059	7.425	7.734	**7.579**	7.164	7.570	**7.367**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.115		0.231		0.326	
CD (5%)	0.326		0.653		0.924	

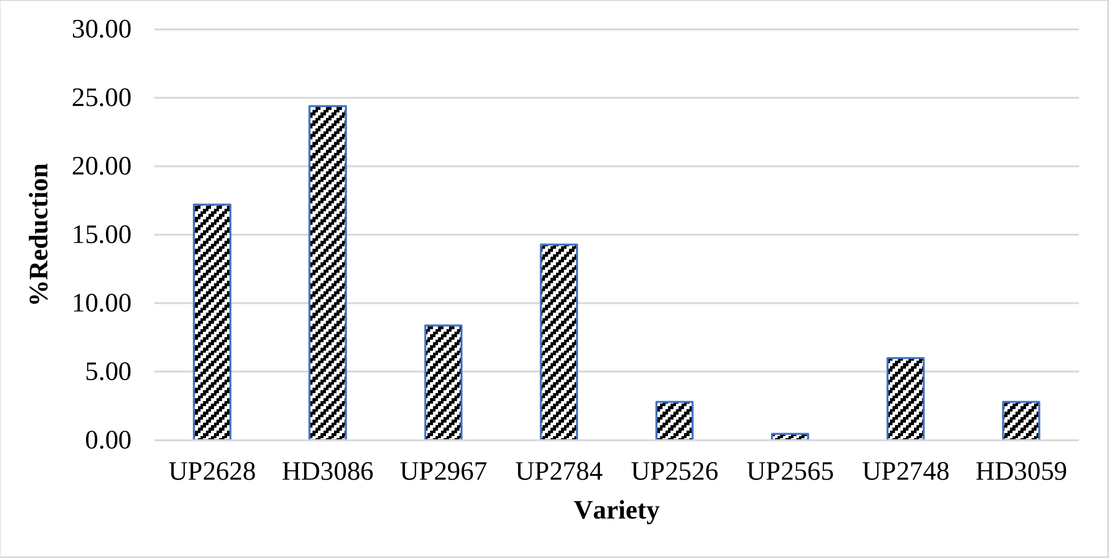


Figure 4.1.6 Reduction in mean values of specific leaf weight of flag leaf of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present research, flag leaf morphology in terms of its fresh weight, dry weight and specific leaf weight was analysed. The results showed reduction in fresh weight, dry weight and specific leaf weight of flag leaves in all varieties under D2 sowing condition in comparison to D1 sowing conditions. Late sowing/in appropriate delay in sowing causes heat stress during growth period of wheat life cycle that affects wheat morphological functions by declining relative growth rate, leaf expansion, fresh weight, dry weight and specific leaf weight **(Nelson, 1998).** The leaves are the site of photosynthetic activity and appear to have an obvious relation to the plant's grain yield ability. Fresh weight and dry weigh of flag leaf is highly corelated with leaf heath and functioning. In an experiment, flag leaf of UP1109 has the highest fresh weight (1.05 grams) and dry weight (0.32 grams) as compared to other wheat varieties under normal condition as under elevated temperature the fresh and dry weight was reduced to 0.93 grams and 0.28 grams respectively. Rest of the wheat varieties namely: PBW396, Raj3765, UP2382, PBW373, C306, VL802, VL738 also showed reduced fresh weight and dry weigh of flag leaves under elevated temperature conditions as compared to normal sown condition. While specific leaf weight was found reduced in UP2382 and C306 under elevated sowing condition as compared to normal sowing condition (**Chandra, 2006).** In previous reports, it was observed that heat stress (due to elevated temperature during anthesis as a result of late sowing) has the widest and most far-reaching effect on water relations leading to severe loss of yield potential of crop. Heat stress causes rapid water loss from the surface of leaf resulting dehydration and sometimes death of plant (**Koini *et al.*, 2009**). The reason for such decrement is due to faster development of growth phases in wheat life cycle (due to heat stress) leads to early senescence which reduced photosynthetic leaf area and fresh weight, dry weight & specific leaf weight during reproductive stage of the crop (**Hatfield, 2015**). A significant negative effect of temperature was observed on specific leaf weight (SLW) and leaf thickness of soybean (Glycine max L. Merril) when 12 soybean genotypes were grown at day/night temperatures of 30/22, 34/24, 38/26 and 42/28 °C with an average temperature of 26, 29, 32 and 35 °C, respectively, under greenhouse conditions which reduces the overall rate of photosynthesis and ultimately yield of the crop (**Jumrani *et al.*, 2017).** Eight wheat genotypes namely Prodip, BARI Gom-25, BARI Gom-26, BAW-1143, BAW-1146, BAW-1147, BAW-1148 and Pavon-76 were analysed under normal late and very late plantation condition. The results showed decrement in leaf area ratio, leaf weight ratio and specific leaf weight under late and very late condition in comparison to normal plantation condition (**Bala and Sikder, 2018**).

4.1.7 Leaf Area Index (LAI)

Leaf area index (LAI) of all the wheat varieties under different sowing condition was calculated during anthesis and the results showed that there was reduction in LAI of wheat varieties under D2 as compared to D1. During 1st trial (2018-19) highest LAI was observed in HD3086 variety (2.77) and lowest in UP2565 variety (1.26) under D1 while under D2 the highest value of LAI was observed in UP2748 variety (1.60) & lowest in UP2565 (1.04). Similarly, during 2nd trial (2019-20), highest LAI was observed in HD3086 variety (2.72) and lowest in UP2565 variety (1.30) under D1 while under D2. the value of LAI was decreased and highest value observed in UP2748(1.63) and lowest in UP2565 (1.04). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value of LAI was achieved by variety HD3086 (2.74) and minimum by UP2565 (1.28) under D1 while under D2 the maximum LAI was recorded in UP2784 variety (1.60) and minimum in UP2565variety (1.04) (**Table 4.1.7**). Over all maximum reduction in LAI due to D2 (elevated temperatures) was observed in HD3086 variety (46.51 %) while minimum reduction was observed in HD3059 variety (4.76%) (**Figure 4.1.7**). Statistically, there was a significant difference in values of leaf area index between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there is non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment and variety, that was found significant ($p \leq 0.01$).

Table 4.1.7 Effect of differential temperatures on leaf area index of different wheat varieties at anthesis. *(D1& D2 indicates sowing in the month of November and December respectively).*

LAI						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	1.82	1.74	**1.78**	1.36	1.40	**1.38**
HD3086	2.77	2.72	**2.74**	1.46	1.47	**1.47**
UP2967	2.46	2.33	**2.39**	1.44	1.41	**1.42**
UP2784	1.88	1.87	**1.88**	1.58	1.63	**1.60**
UP2526	1.75	1.74	**1.75**	1.57	1.50	**1.53**
UP2565	1.26	1.30	**1.28**	1.04	1.04	**1.04**
UP2748	1.94	1.86	**1.90**	1.60	1.51	**1.55**
HD3059	1.68	1.62	**1.65**	1.56	1.58	**1.57**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.024		0.048		0.067	
CD (5%)	0.067		0.135		0.191	

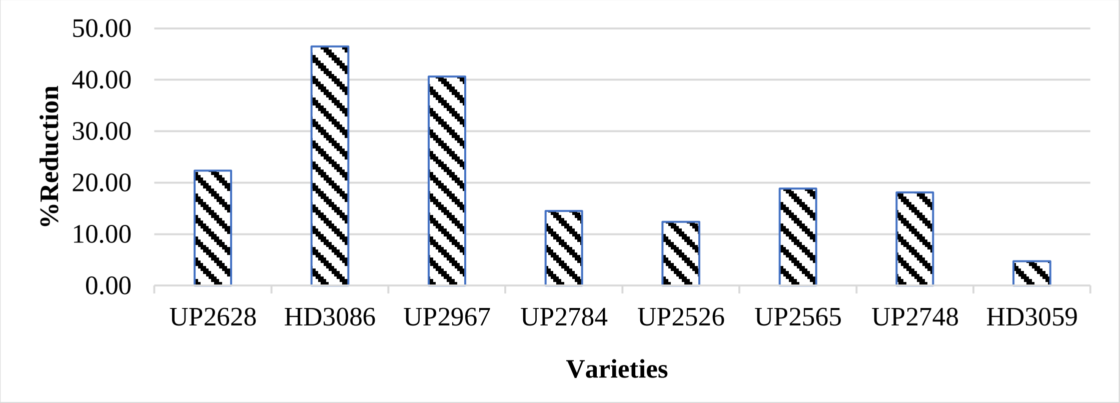


Figure 4.1.7. Reduction in mean values of leaf area index of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

Flag leaf is responsible for controlling the plant growth and productivity by influencing the dry matter accumulation in various crop species (**Potter and Jones, 1997).** It was reported that the optimum sowing wheat (16th November) has the highest values for LAI (Leaf Area Index) and dry matter accumulation at all the growth stages (tillering, anthesis and at maturity. But under in appropriate sowing (26th November) LAI was found gradually decreased. In this research Sonalika wheat genotype showed gave the highest values of LAI and also recorded greater number of ears per m^2 (319), grains per ear (27.3) and test weight (39.49 g) than other wheat variety (UP262) (**Ghosh *et al.* 2000).** The reason for such decrement is that high temperature during anthesis leads to forced maturity in wheat crop which reduced leaf area and leaf area index **(Kanchan and Mahendra, 2000.).** Similar trend also followed by (**Sharma *et al.* (2000) and (Verma *et al*., 2001).** According to one research under high temperature stress reduction in leaf area index and leaf width was also observed in different wheat varieties **(Singh *et al*., 2001).** In previous researches, it was found that the flag leaf is very sensitive towards elevated temperature results reduced assimilate supplies to ear head and reduced grain weight & yield (**Dash and Mohanty, 2001**). An experiment was conducted at Karnataka found that wheat genotype DWR 195 had recorded higher leaf area index followed by DWR162, DWR 185 and DWR 1013 under normal sown condition in comparison to late sown condition **(Prabhakar *et al*., 2002)**. Some studies also showed that under November 22 sown condition, higher leaf area index was observed in wheat varieties such as PBW343 and UP2425 while same varieties under December 22 sown conditions, LAI was reduced (**Mishra, 2002).** Similar trend also followed by other scientists in which delay of sowing in wheat decreased the leaf area index of flag leaf at all growth stages significantly (**Singh and Pal, 2003).** The reason for such reduction is due to some anatomical changes takes place in plants under elevated temperature due to inappropriate sowing such as reduction in cell size, partial closure of stomata for restriction towards water loss, increased number of xylem vessels in roots and shoots that lead to have negative effect on growth and development of plant (**Anonymous, 2004**). A research was conducted on heat tolerant and heat sensitive wheat genotypes grown under normal and elevated temperatures which revealed that leaf area index was significantly decreased in heat sensitive genotypes as compared to heat tolerant genotypes grown under elevated temperatures (**Dhyani *et al.*, 2013**). Through recent research, Heat and drought + heat was found to have a much greater influence on the leaf area index. Through an experiment in wheat variety (Faisalabad-2008), it is concluded that under controlled condition (no stress) LAI was 3.31 ± 0.191 while under heat treated condition LAI was reduced to 2.17 ± 0.090 and with the increment of stress (drought + heat) LAI reduced to 1.92 ± 0.092. This shows that LAI have negative relation with leaf area index **(Sattar *et al.*, 2020**).

4.1.8 Total dry matter (TDM)

Total dry matter of different wheat varieties was recorded at the time of anthesis and maturity under both sowing conditions (D1 and D2). From the results it was observed that under D2condition total dry matter of all the wheat varieties was reduced in comparison to D1 during anthesis as well as during maturity. At anthesis, during 1st trial (2018-19) highest TDM was observed in UP2628 variety (14.45 grams) and lowest in UP2748 variety (11.16 grams) underD1 while underD2, TDM was reduced and the highest value was observed in UP2628 (12.83 grams) & lowest in UP2784 variety (10.92grams). Similarly, during 2nd trial (2019-20), highest TDM was observed in UP2784 (14.80 grams) and lowest in UP2748 (11.10 grams) under D1 and under D2 highest value of TDM was observed in UP2967 (12.43 grams) and lowest in UP2526 variety (10.08 grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value for TDM was achieved by variety UP2628 (14.22 grams) and minimum by UP2748 (11.33 grams) under D1 while under D2 condition, the maximum TDM was recorded in UP2967 variety (12.60 grams) and minimum in UP252 6variety (10.68 grams) (**Table 4.1.8**). Over all maximum reduction in TDM due to D2 (elevated temperatures) was observed in UP2784 variety (20.49%) while minimum reduction was observed in UP2565 variety (1.24 %) (**Figure 4.1.8**). Statistically, there was a high significant difference in values of total dry matter between both sowing conditions (D1and D2) and between varieties ($p \leq 0.001$) while there is non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other($p > 0.05$) except the interaction between treatment and variety, that was found significant ($p \leq 0.001$).

At maturity, during 1st trial (2018-19) highest TDM was observed in HD3059 variety (28.17 grams) and lowest in HD3086 variety (20.22 grams) under D1 while under D2, TDM was reduced and the highest value was observed in HD3059 variety (24.60 grams) & lowest in UP2784 variety (16.33 grams). Similarly, during 2nd trial (2019-20), highest TDM was observed for UP2628 variety (26.84 grams) and lowest in UP2565 (20.70 grams) under D1 while under D2 highest value of TDM was observed for HD3059 variety (24.80 grams) and lowest for UP2784 variety (16.27 grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value of TDM was achieved by variety HD3059 (27.01 grams) and minimum by UP2526 (20.47 grams) under D1 while under D2, the maximum TDM was recorded for HD3059 variety (24.70 grams) and minimum for UP2784 variety (16.30 grams) (**Table 4.1.8**). Over all maximum reduction in TDM due to D2 (elevated temperatures) was observed in UP2784 variety (39.50 %) while minimum reduction was observed in HD3059 variety (8.56 %) (**Figure 4.1.8**). Statistically there was a high significant difference in values of total dry matter between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.001$) while there is non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other($p > 0.05$) except the interaction between treatment and variety, that was found significant ($p \leq 0.001$).

Table 4.1.8 Effect of differential temperatures on total dry matter (grams) of different wheat varieties at anthesis and maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

Total Dry Matter (grams)												
	At anthesis						At maturity					
Varieties	D1			D2			D1			D2		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
UP2628	14.45	14.00	**14.22**	12.83	11.48	**12.16**	25.60	26.84	**26.22**	21.30	20.76	**21.03**
HD3086	13.66	13.76	**13.71**	11.48	11.63	**11.56**	20.22	22.67	**21.44**	17.53	17.97	**17.75**
UP2967	12.91	12.82	**12.86**	12.77	12.43	**12.60**	22.85	21.02	**21.93**	17.30	17.51	**17.41**
UP2784	13.53	14.80	**14.17**	10.92	11.60	**11.26**	27.21	26.67	**26.94**	16.33	16.27	**16.30**
UP2526	11.36	11.66	**11.51**	11.29	10.08	**10.68**	26.09	25.35	**25.72**	23.20	22.40	**22.80**
UP2565	11.41	11.78	**11.59**	11.22	11.68	**11.45**	20.23	20.70	**20.47**	17.93	17.30	**17.62**
UP2748	11.16	11.10	**11.13**	11.07	10.61	**10.84**	25.73	25.00	**25.37**	22.13	21.58	**21.86**
HD3059	12.13	12.03	**12.08**	11.19	11.38	**11.29**	28.17	25.86	**27.01**	24.60	24.80	**24.70**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.106		0.212		0.300		0.158		0.316		0.446	
CD (5%)	0.300		0.600		0.848		0.446		0.893		1.265	

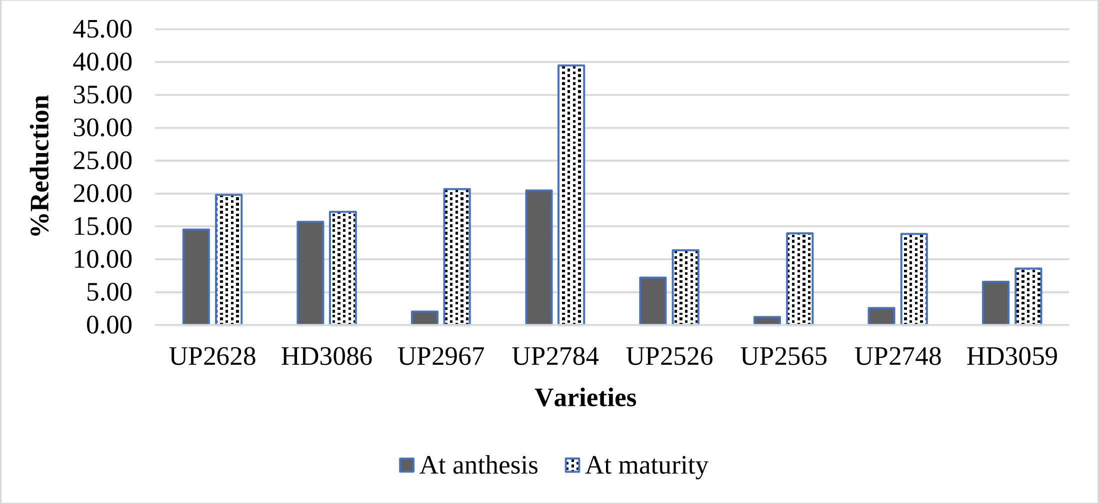


Figure 4.1.8. Reduction in mean values of total dry matter of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

As flag leaf is responsible for controlling plant growth and productivity it is concluded that if the leaf area index decreases under D2 condition, total dry matter also negatively affected (**Potter and Jones, 1997).** Other experiments also found that in wheat variety HD2189 has recorded significantly higher dry matter production (632.96 gm/m^2) over HD4502 and HD2281 under non--stress as compared to elevated temperatures (**Srinivas, 1999**). It is reported that when wheat varieties sown at 16th November, has the highest values for dry matter accumulation at all the growth stages (tillering, anthesis and maturity) in comparison to when wheat varieties at the month of December (**Ghosh *et al.* 2000)**. But through various researches it is well known that flag leaf is most affected by late plantation (elevated temperatures) as in wheat, elevation in temperatures causes reduced assimilate supplies to ear head and dry matter accumulation (**Dash and Mohanty, 2001**). In the present research also LAI of different wheat varieties was found reduced under elevated temperature similarly dry matter (TDM) also found to be decreased due to heat stress conditions. The reason for such decrement in dry matter accumulation under delay in sowing condition is that during optimum sowing condition there is enhanced vegetative phase and more radiation absorption as compared to delay in sowing, where forced maturity takes due to higher temperature led to completion of phenological stages quickly by reducing the duration hence late sowing condition has TDM that was found reduced for some varieties. Other scientists also reported that normal sown crop produced significantly more dry matter than late seeded crops (**Shivani *et al.*, 2001**). This result confirms findings of (**Verma *et al*., 2001**). An experiment has conducted at Karnataka and found that wheat genotype DWR 195 recorded higher dry matter accumulations (288.58 g/m row length) and leaf area(83.44 dm^2/m^2) followed by DWR162, DWR185 and DWR 1013 under optimum sown condition as compared to late sown condition (**Prabhakar *et al*., 2002**). According to one of the studies in wheat variety UP2565, which was under three different sowing dates i.e. 1st December, 20th December and 6th January, the results concludes that leaf area index and dry matter was significantly affected by sowing dates. The crop sown under late sown condition took a smaller number of days to attain maturity in comparison to the crop sown normal causes reduction in leaf area index as well as dry matter accumulation as the sowing dates increases. The maximum reduction in dry matter was found for the crop sown on 6th December. The reason for this decrement according to the author was the sudden elevated temperature at the reproductive stage during the crop growing season (**Gupta, 2017**). An experiment conducted with two winter barley cultivars (Cordoba and Greval) under different sowing dates of month (September and October) for two successive years. The results showed that October sowing barley cultivars has led to a significant decrease in the maximum value of plant dry weight accumulation while September sown barley achieves higher plant dry matters accumulation which is one of the main determinants of grain yield (**Mirosavljević *et al.*, 2018**).

4.1.9 Inter-nodal Distance (1^{st}, 2^{nd} and 3^{rd}) (From Top to Base)

At the time of maturity, inter-nodal distance (from top to base) of wheat varieties under both sowing conditions were recorded. The results showed that in all wheat varieties 1^{st} internode has the maximum length followed by 2^{nd} and 3^{rd}. From data it can also be analysed that out of 1^{st} 2^{nd} and 3^{rd} internodal distance, 3^{rd} internode is most affected by D2 (elevated temperatures) with maximum percent reduction (up to16.81%) in comparison to 1^{st} (up to 13.93%) and 2^{nd} internodal distance (up to 9.54). As in detail view, during 1^{st} trial (2018-19) maximum distance of 1^{st} internode was observed in UP2526 variety (22.33cm) and minimum in UP2748 variety (18.00 cm) under D1 while under D2, the distance of 1^{st} internode was found reduced and the maximum was observed in UP2526 (21.00 cm) and minimum in UP2628 (16.00 cm). Similarly, during 2^{nd} trial (2019-20), maximum 1^{st} internodal distance was observed for HD3086 variety (22.11 cm) and minimum in HD3059 (18.00 cm) under D1 while under D2 highest value of1^{st} internodal distance was observed for UP2526 variety (20.33 cm) and lowest for UP2628 variety (16.90 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value for 1^{st}internodal distance within varieties was achieved by variety UP2526 (22.00 cm) and minimum by UP2748 (18.22 cm) under D1 while under D2, the maximum distance of 1^{st} internodal distance was recorded for UP2526 variety (20.67 cm) and minimum for UP2628 variety (16.45 cm) (**Table 4.1.9**). Over all maximum reduction in 1^{st} internodal distance due to D2 (elevated temperatures) was observed in UP2628 variety (13.93 %) while minimum in UP2748 variety (5.49%) (**Figure 4.1.9**). Statistically, data concludes that there was a significant difference in values of 1^{st} internodal distance between both sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there is non-significant difference was observed between the two years data ($p > 0.05$). All the other interactions were also found non-significant with each other(p>0.05) except the interaction between year and variety, that was found significant ($p \leq 0.001$).

For 2nd internodal distance, during 1st trial (2018-19) maximum distance of 2nd internode was observed in UP2526 variety (20.44 cm) and minimum in UP2628 variety (17.29 cm) underD1 condition while under D2 the distance of 2nd internode was found reduced and the maximum value was observed in UP2526 (19.00 cm) and minimum in UP2628 variety (16.00 cm). Similarly, during 2nd trial (2019-20), maximum 2nd internodal distance was observed for UP2526 variety (20.00 cm) and minimum in UP2967 and UP2748(17.22 cm) under D1 while under D2 highest value of2nd internodal distance was observed for UP2526 variety (18.22 cm) and lowest for UP2748 variety (15.67 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value for 2nd internodal distance within varieties was achieved by variety UP2526 (20.22 cm) and minimum by UP2748 (17.28 cm) under D1 while under D2, the maximum 2ndinternodal distance was recorded for UP2526 variety (18.61cm) and minimum for UP2628 variety (15.95 cm) (**Table 4.1.9**). Over all maximum reduction in 2nd internodal distance due to D2 (elevated temperatures) was observed in UP2784 variety (9.54 %) while minimum in UP2565 variety (6.63%) (**Figure 4.1.9**). Statistically, data concludes that there was a high significant difference in values of 2nd internodal distance between both sowing conditions (D1and D2) and between varieties ($p \leq 0.001$) There is also a significant difference was observed between the two years data ($p \leq 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$).

For 3rd internodal distance, during 1st trial (2018-19) maximum distance of 3rd internode was observed in UP2526 variety (18.52 cm) and minimum in UP2628 variety (12.89 cm) under D1 condition while under D2, the distance of 3rd internode was found reduced and the maximum value was observed in UP2526 (16.50 cm) and minimum value observed in UP2628 variety (11.00 cm). Similarly, during 2nd trial (2019-20), maximum 3rdinternodal distance was observed for UP2526 variety (18.55 cm) and minimum in UP2628 (13.56 cm) under D1 while under D2, highest value of 3rd internodal distance was observed for UP2526 variety (16.80 cm) and lowest for UP2628 variety (11.00 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it is concluded that maximum value for 3rd internodal distance within varieties was achieved by variety UP2526 (18.54 cm) and minimum by UP2628 (13.22 cm) under D1 while under D2, the maximum 3rdinternodal distance was recorded again for UP2526 variety (16.65 cm) and minimum for UP2628 variety (11.00 cm) (**Table 4.1.9**). Over all maximum reduction in 3rd internodal distance due to D2 (elevated temperatures) was observed in UP2628 variety (16.81 %) while minimum in HD3059 variety (7.65%) (**Figure 4.1.9**). Statistically, there was a high significant difference in values of 3rd internodal distance between both sowing conditions (D1and D2), between varieties as well as between two years ($p \leq 0.001$). All the other interactions were also found non-significant with each other($p > 0.05$) except the interaction between year and variety, that was found significant ($p \leq 0.05$).

Table 4.1.9 Effect of differential temperatures on Inter- nodal distance in cm (From Top to Base) of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

Varieties	Inter- nodal distance (cm) (From Top to Base)						
		D1			D2		
	Internode	2018-19	2019-20	Mean	2018-19	2019-20	Mean
UP2628	1st	18.78	19.44	**19.11**	16.00	16.90	**16.45**
	2nd	17.29	17.78	**17.54**	16.00	15.89	**15.95**
	3rd	12.89	13.56	**13.22**	11.00	11.00	**11.00**
HD3086	1st	21.00	22.11	**21.55**	19.00	19.55	**19.28**
	2nd	19.67	18.11	**18.89**	17.00	17.33	**17.17**
	3rd	16.89	15.33	**16.11**	14.33	13.22	**13.78**
UP2967	1st	18.89	19.00	**18.94**	16.56	16.78	**16.67**
	2nd	19.11	17.22	**18.17**	16.99	16.11	**16.55**
	3rd	17.89	16.22	**17.06**	14.89	14.44	**14.67**
UP2784	1st	20.67	19.89	**20.28**	19.33	18.56	**18.95**
	2nd	18.44	17.67	**18.06**	16.00	16.66	**16.33**
	3rd	15.89	14.67	**15.28**	14.00	12.80	**13.40**
UP2526	1st	22.33	21.67	**22.00**	21.00	20.33	**20.67**
	2nd	20.44	20.00	**20.22**	19.00	18.22	**18.61**
	3rd	18.52	18.55	**18.54**	16.50	16.80	**16.65**
UP2565	1st	20.33	20.33	**20.33**	19.00	19.11	**19.05**
	2nd	19.11	18.67	**18.89**	18.11	17.17	**17.64**
	3rd	17.89	16.74	**17.32**	15.78	15.44	**15.61**
UP2748	1st	18.00	18.44	**18.22**	17.44	17.00	**17.22**
	2nd	17.33	17.22	**17.28**	16.44	15.67	**16.06**
	3rd	16.33	15.55	**15.94**	15.00	14.00	**14.50**
HD3059	1st	19.22	18.00	**18.61**	17.00	17.22	**17.11**
	2nd	18.44	17.33	**17.89**	17.00	16.00	**16.50**
	3rd	16.11	15.89	**16.00**	15.00	14.56	**14.78**
		Treatment (T)		**Variety (V)**		**(TXV)**	
1st	**SEm ±**	0.121		0.235		0.344	
	CD (5%)	0.344		0.688		0.973	
2nd	**SEm ±**	0.126		0.252		0.357	
	CD (5%)	0.357		0.714		1.010	
3rd	**SEm ±**	0.107		0.214		0.303	
	CD (5%)	0.303		0.606		0.858	

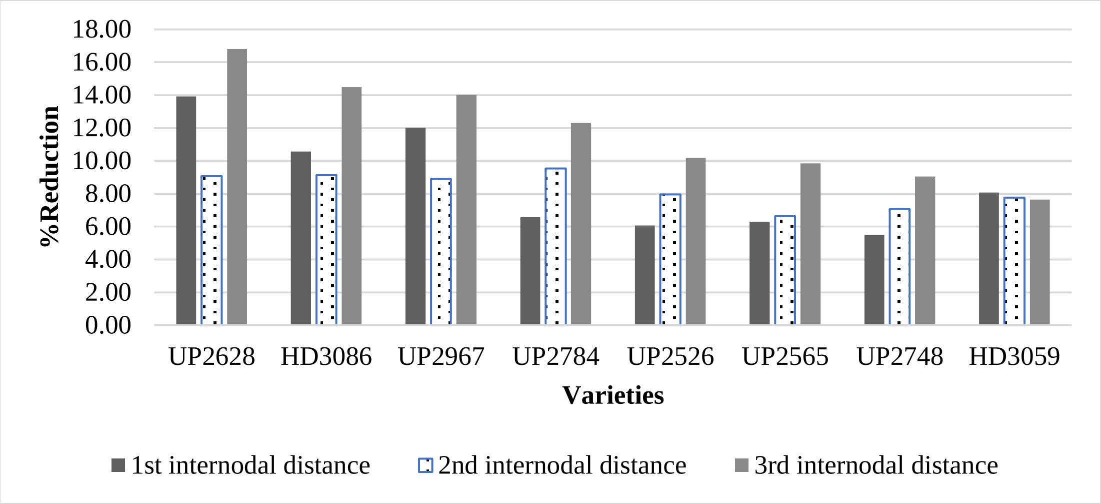


Figure 4.1.9 Reduction in mean values of inter-nodal distance (Top to Base) of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

High temperature prevailing during anthesis leads to force maturity, reduces internodal distance when wheat plant grown under elevated temperatures (**Kanchan and Mahendra, 2000**). Similar trend also followed by present research. In contrast, according to one research, under high temperature stress internodal distance sometimes also found increase **(Singh *et al*., 2001).** Maintenance of internodal distance is also associated with plant height, previous research shows that reduction in plant height due to heat stress is mainly due to reduced differential loss of internodal length of various crop species. First internodal distance seems to be more affected by delayed sowing however in contrast, increase in internodal distance also observed in some crop species such as sugar cane and wheat under elevated temperatures condition (**Singh and Singh, 2000).** Similar trend also followed by some other scientists, as in an experiment with different wheat varieties under different sowing conditions observed that internodal distance significantly affected by sowing timing and cultivars (under heat stress condition) have shorter internodal distance as compared to non-stress condition cultivars. From this experiment it can be concluded that maximum reduction due elevation in temperature on 1st internode was 37.14 %. Maximum reduction on 2nd internode was found 34.4% and maximum reduction on 3rd internode was observed 34.40% for variety UP2113 **(Srivastava, 2005**). As under heat stress conditions, internodal distance of wheat varieties were found reduces, according to some researches it could be beneficial for controlling lodging. An experiment was conducted in which two winter wheat cultivars (Tainong 18 and Shannong 15) were sown on three dates (October 1, October 8, and October 15). The results showed that as the sowing dates increases internodal distance decreases and plant height of the varieties reduced. As the lodging is concerned, there was no significant differences in lodging resistance was observed for first two sowing dates but due to postponing the sowing dates the lodging resistance in wheat varieties were significantly increased by 53.6% and 49.6%. The reason for increased lodging resistance was mainly through a reduction in the culm height at the center of gravity and an increase in the tensile strength of the base internode under delay in sowing dates (**Dai *et al*., 2017**). Similar trend also followed by other scientists in which inter-nodal distance in wheat varieties (PBW396, VL738, C306 and PBW373) were found highly affected by date of sowing, as late sown cultivars have shorter distance compared to normal sown cultivars. 1st internode was found most affected in this case study and maximum reduction due to elevated temperatures (late sowing) was observed in PBW-396 (37.02%) **(Chandra and Srivastava, 2020).**

Correlation analysis between morphological parameters with grain yield under both sowing condition

A positive and significant correlation was observed by taking the average of two years data, under D1 between grain yield and four variables; plant height (r=0.79*), tiller number (0.92**), productive tillers (0.79*) and total dry matter (r=0.90**) while under D2, no such significant positive correlation was observed between grain yield and other morphological parameters. On comparing between morphological traits, positive and significant correlation was observed for total dry matter with other variables such as plant height (r=0.91**) tiller numbers (0.94**) and productive tillers (0.61) under D1. However, a positive correlation was also found between tiller number and productive tillers (r=0.72*) under D2. The study concludes that due to sowing done in month of December (elevated temperatures), the relationship between grain yield with other morphological traits were highly affected, may be due to the reduced grain yield under D2 in comparison to D1. Under D1, more positive and significant correlations between morphological traits were observed in comparison to D2. In present study in some wheat varieties least reduction in some traits was found under D2 (elevated temperatures) may be due to absence of negative correlation between parameters were observed which was significant (**Table 4.1.10.1 and Table 4.1.10.2).**

Table 4.1.10.1 Pearson's correlation between morphological parameters with grain yield under D1.

	GY	*PH*	*TN*	*PT*	*FLA*	*LAI*	*SLW*	*TDM*
GY	1							
PH	0.79*	1						
TN	0.92**	0.85**	1					
PT	0.79*	0.63	0.63	1				
FLA	-0.65	-0.37	-0.40	-0.43	1			
LAI	0.09	0.64	0.26	0.09	0.08	1		
SLW	-0.32	-0.21	-0.23	-0.62	-0.05	-0.05	1	
TDM	0.90**	0.91**	0.94**	0.61	-0.51	0.44	-0.27	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Plant height (PH), Tiller number (TN), Productive tillers (PT), Flag leaf area (FLA), Leaf area index (LAI), Specific leaf weight (SLW) and Total dry matter (TDM).

Table 4.1.10.2 Pearson's correlation between morphological parameters with grain yield under D2.

	GY	*PH*	*TN*	*PT*	*FLA*	*LAI*	*SLW*	*TDM*
GY	1							
PH	-0.21	1						
TN	0.49	-0.14	1					
PT	0.36	0.11	0.72*	1				
FLA	-0.37	-0.44	0.17	0.17	1			
LAI	-0.43	-0.08	-0.42	-0.56	-0.33	1		
SLW	-0.45	0.29	-0.47	-0.25	-0.16	0.31	1	
TDM	0.26	0.29	-0.41	0.01	-0.35	-0.31	-0.26	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Plant height (PH), Tiller number (TN), Productive tillers (PT), Flag leaf area (FLA), Leaf area index (LAI), Specific leaf weight (SLW) and Total dry matter (TDM).

4.2 Effect of elevated temperatures on Physiological Characteristics;

4.2.1 Relative water content (RWC)

Relative water content of different wheat varieties was calculated at the anthesis and 15 days after anthesis (15 DAA) in two consecutive years 2018-19 and 2019-20. RWC was found to be reduced due to D2 (elevated temperatures) in both years at anthesis as well as 15 DAA (grain filling) as compared to D1 and found higher at the time of anthesis in comparison to grain filling stage (DAA). As the varieties tends towards grain filling, RWC found reduced under both sowing conditions (D1 and D2). At anthesis, during 1st trial (2018-19), highest value of RWC was observed in HD3086 (89.71%) and lowest in HD3059 (77.67 %) under D1 while under D2, highest value recorded in UP2967 variety (64.25 %) and lowest in UP2526 (44.97 %). Similarly, during 2nd trial (2019-20), highest RWC was observed in HD3086 variety (88.75 %) and lowest in UP2967 (74.74 %) under D1 while under D2, highest RWC was observed in UP2565 (63.13 %) and lowest in UP2748 (53.27 %). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at anthesis maximum RWC was attained by HD3086 variety (89.23 %) and minimum by UP2967 (76.28 %) under D1. However, under D2, maximum RWC was attained for UP2967 variety (63.51 %) and minimum for UP2526 (45.04 %) (**Table 4.2.1**). Over all at the time of anthesis maximum reduction in RWC due to D2 (elevated temperatures) was observed in UP2526 (48.05 %) while minimum in UP2967 (16.73 %) (**Figure 4.2.1**). Statistically, there was a significant difference in RWC values at the time of anthesis between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found significant with each other ($p \leq 0.01$) except the interactions between year × treatment as well as for year × treatment × varieties, that was found non-significant ($p > 0.05$).

At grain filling stage (15 DAA), during 1st trial (2018-19), highest value of RWC was observed in UP2628 (72.67 %) and lowest in UP2967 and UP2748 (55.33 %) under D1 while under D2, RWC was found to be reduced & highest value was observed in UP2565 variety (56.04%) and lowest in UP2628 (37.43 %). Similarly, during 2nd trial (2019-20), highest RWC was observed in UP2628 variety (71.26 %) and lowest in UP2967 (54.74 %) under D1 while under D2, highest RWC was observed in HD3059 (58.00 %) and lowest in UP2628 (31.09 %). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at grain filling stage (15 DAA) maximum RWC was attained by UP2628 variety (71.97 %) and minimum by UP2967 (55.04 %) under D1. However, under D2 maximum RWC was attained for UP2565 variety (53.48 %) and minimum for UP2628 (34.26 %) (**Table 4.2.1**). Over all at the time of grain filling (15 DAA) maximum reduction in RWC due to D2 was observed in UP2628 (52.39 %) while minimum in UP2967 (4.18 %) (**Figure 4.2.1**). Statistically, RWC under each year, treatment and variety concludes that there was a significant difference in RWC values at the time of anthesis between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found significant with each other ($p \leq 0.01$).

Table 4.2.1 Effect of differential temperature on relative water content (%) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

Relative Water Content (%)												
	At anthesis						**15 DAA**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	82.11	81.26	**81.69**	56.35	58.72	**57.54**	72.67	71.26	**71.97**	37.43	31.09	**34.26**
HD3086	89.71	88.75	**89.23**	63.95	62.33	**63.14**	55.88	57.21	**56.55**	42.00	41.37	**41.69**
UP2967	77.81	74.74	**76.28**	64.25	62.78	**63.51**	55.33	54.74	**55.04**	53.80	51.67	**52.73**
UP2784	82.77	80.52	**81.65**	55.89	54.48	**55.19**	58.67	60.52	**59.59**	50.63	52.64	**51.63**
UP2526	87.26	86.14	**86.70**	44.97	45.12	**45.04**	66.33	65.91	**66.12**	39.77	38.18	**38.98**
UP2565	82.98	84.89	**83.94**	62.17	63.13	**62.65**	63.67	66.87	**65.27**	56.04	50.93	**53.48**
UP2748	80.19	79.92	**80.06**	53.30	53.27	**53.28**	55.33	55.24	**55.29**	49.90	45.67	**47.78**
HD3059	77.67	79.04	**78.35**	61.78	62.60	**62.19**	56.57	65.49	**61.03**	45.22	58.00	**51.61**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.221		0.442		0.625		0.226		0.452		0.639	
CD (5%)	0.625		1.250		1.769		0.639		1.278		1.808	

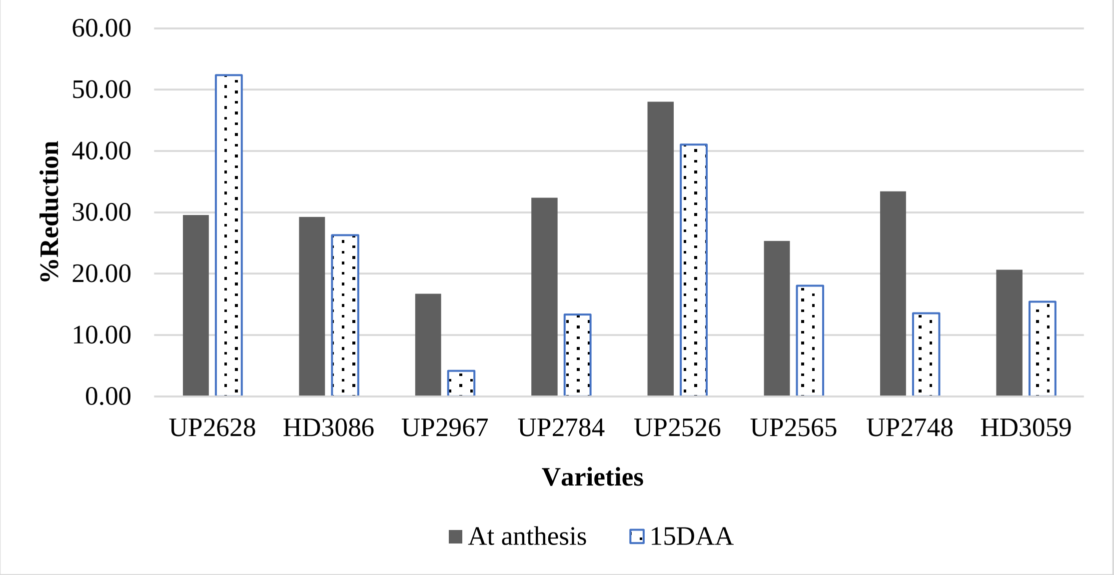


Figure 4.2.1 Reduction in mean values of relative water content of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In investigated research RWC was found to be reduced under D2 in comparison to D1. At anthesis, RWC was also found higher in all wheat varieties as compared to their grain filling stage. From previous study, when three different wheat varieties (C306, HD2285 and HD2329) were superimposed to elevated temperature (heat stress). Delay in sowing timings showed similar trend in which the RWC was found significantly and negatively affected by D2 (elevated temperature condition). On comparing between varieties, C306 had good RWC under both sowing condition and the reduction was also less as compared to other varieties. So out of three wheat varieties, C306 could be considered as medium behavioural wheat variety which is suitable for both sowing condition while rest of the varieties showed significant reduction in RWC under elevated temperatures and considered as susceptible towards heat stress (**Sairam *et al*., 2000).** According to previous other findings, high temperature causes reduced water availability in plants. Under elevated temperatures there is high vapor pressure deficit condition along with high evapotranspiration which leads to decrease relative water content along with water potential in leaves of plant. In previous researches also it was found that under late plantation RWC of many crop species was reduced, especially in wheat, as it a cool season crop and due to late sowing, it experiences higher temperature during anthesis and grain filling which are considered as most sensitive towards temperature leads to reduction in RWC at that time (**Maazorra *et al*., 2002).** Similar results also recorded by some scientists in which RWC was significantly reduced at anthesis and after anthesis when the two wheat genotypes exposed to elevated temperature conditions (**Almenselman *et al*., 2009).** In an investigation, relative water content in different wheat varieties was significantly affected by elevated temperatures during grain filling (15 DAA) as compared to anthesis stage. During anthesis stage, the difference (upto 4%) in RWC was observed in varieties between optimum and late sowing which was found non-significant. As RWC is an adaptive phenomenon so wheat varieties (Raj4101, Raj 376 and Lok540) are tolerant genotypes as they showed higher RWC during elevated temperatures. The reason for the reduction of RWC, is that due to heat stress/high temperature, sudden increase in transpiration occurs and the tendency of plants to maintain its tissue water status is impaired resulting decrement in RWC in the leaves. **(Dhyani, 2010).** In a study, two wheat varieties (Sehar 2006 and Faislabad 2008) were sown on three different dates (10th Nov, 10th Dec and 10th Jan) for two consecutive years 2011–2012 and 2012–2013 to check the effect of sowing timings on physiological responses and water status of wheat varieties under elevated temperatures. They conclude that high temperature due to delay in sowing dates reduce the water relations related parameters. The relative water contents were significantly reduced due to elevated temperatures in wheat. Maximum RWC was noted in 10th November sowing timing. Their data revealed that water potential and osmotic potential became more negative due to late planting of wheat **(Sattar *et al*., 2017).** In another study relative water content in wheat genotypes (Salt-6, Lu-26s, TJ-83, RN-09111, KIRAN-95, NRL-1236 and NIA-AS-14-10) was reduced up to 80% under elevated temperatures condition as compared to optimum condition in which RWC was above 95% in all wheat varieties (**Khan *et al*., 2020**). In a study, wheat variety (Faisalabad-2008) was grown under different conditions: controlled (open space under environmental normal condition) and heat stressed (wheat grown inside the plastic tunnel during reproductive phase). The highest percentage of RWC was observed in control condition whereas under heat stress notably decreased RWC by 25 % was observed in comparison to control (**Sattar *et al*., 2020**).

4.2.2 Membrane stability Index (MSI)

Membrane stability index of different wheat varieties was also reported at the anthesis and 15 days after anthesis (15 DAA) in two consecutive years 2018-19 and 2019-20. MSI was reduced under D2 (elevated temperatures) in both years at anthesis as well as 15 DAA (grain filling) as compared to D1. As due to heat stress the stability of membrane was found disturbed in comparison to the membrane of wheat varieties under normal condition. On comparing between the stages, MSI in all wheat varieties was also found higher at the time of anthesis as compared to grain filling stage (15DAA). As the varieties tends towards grain filling, MSI was found reduced under both sowing conditions (D1 and D2). At anthesis, during 1st trial (2018-19), highest value of MSI was observed in UP2565 (57.54 %) and lowest in UP2967 (34.65 %) under D1 while under D2, MSI was found to be reduced and highest value observed in UP2565 variety (24.83 %) and lowest in UP2748 (15.00 %). Similarly, during 2nd trial (2019-20), highest MSI was observed in UP2565 variety (57.80 %) and lowest in UP2967 (34.24 %) under D1 while under D2, highest MSI was recorded in UP2628 (27.92 %) and lowest in UP2748 (14.21 %). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at anthesis, maximum MSI was attained by UP2565 variety (57.67 %) and minimum by UP2967 (34.45 %) under D1. However, under D2, maximum for UP2628 variety (24.18 %) and minimum for UP2748 (14.61%) (**Table 4.2.2**). Over all at the time of anthesis, maximum reduction in MSI due to D2 (elevated temperatures) was observed in UP2748 (66.22 %) while minimum in in UP2967 (36.31%) (**Figure 4.2.2**). Statistically, under each year, treatment and variety concludes that there was a significant difference in RWC values at the time of anthesis between the two-sowing conditions (D1 and D2) and between varieties as well as between years ($p \leq 0.01$). All the interactions were also found significant with each other ($p \leq 0.01$).

At grain filling (15 DAA), during 1st trial (2018-19), highest MSI was observed in UP2784 (42.24 %) and lowest in HD3086 (25.02 %) under D1 while under D2, MSI was found to be reduced and highest value observed in HD3086 variety (17.20 %) and lowest in UP2748 (10.09 %). Similarly, during 2nd trial (2019-20), highest MSI was observed in UP2784 variety (45.80 %) and lowest in HD3086

(27.63 %) under D1 while under D2, highest MSI was observed in UP2628 (19.05 %) and lowest in UP2565 (11.22 %). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at grain filling, maximum MSI was attained by UP2784 variety (44.02 %) and minimum by HD3086 (26.33 %) under D1. However, under D2, maximum MSI was attained for UP2628 variety (17.16 %) and minimum for HD3059 (11.72 %) (**Table 4.2.2**). Over all at the time of grain filling the maximum reduction in MSI due to D2 (elevated temperatures) was observed in UP2565 (70.65 %) while minimum in HD3086 (35.25 %) (**Figure 4.2.2**). Statistically. under each year, treatment and variety, there was a significant difference in RWC values at the time of grain filling between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found significant with each other ($p \leq 0.01$) except the interactions between year × treatment as well as for year × treatment × varieties, that was found non-significant ($p > 0.05$).

Table 4.2.2 Effect of differential temperatures on membrane stability index (%) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

Membrane Stability Index (%)												
	At anthesis						**15 DAA**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	42.33	47.24	**44.78**	20.44	27.92	**24.18**	33.78	35.52	**34.65**	15.28	19.05	**17.16**
HD3086	41.03	43.69	**42.36**	20.45	19.12	**19.78**	25.02	27.63	**26.33**	17.20	16.89	**17.05**
UP2967	34.65	34.24	**34.45**	24.75	19.13	**21.94**	32.58	30.03	**31.30**	15.77	14.24	**15.01**
UP2784	55.91	53.15	**54.53**	20.30	18.79	**19.54**	42.24	45.80	**44.02**	16.57	15.13	**15.85**
UP2526	50.50	43.36	**46.93**	18.20	15.46	**16.83**	31.82	38.23	**35.03**	16.52	13.74	**15.13**
UP2565	57.54	57.80	**57.67**	24.83	16.03	**20.43**	40.84	40.50	**40.67**	12.65	11.22	**11.93**
UP2748	40.76	45.71	**43.23**	15.00	14.21	**14.61**	28.22	28.79	**28.51**	10.09	13.52	**11.81**
HD3059	42.67	45.78	**44.23**	17.71	15.86	**16.78**	35.55	28.65	**32.10**	11.75	11.69	**11.72**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.138		0.276		0.390		0.208		0.417		0.590	
CD (5%)	0.390		0.780		1.103		0.589		1.179		1.668	

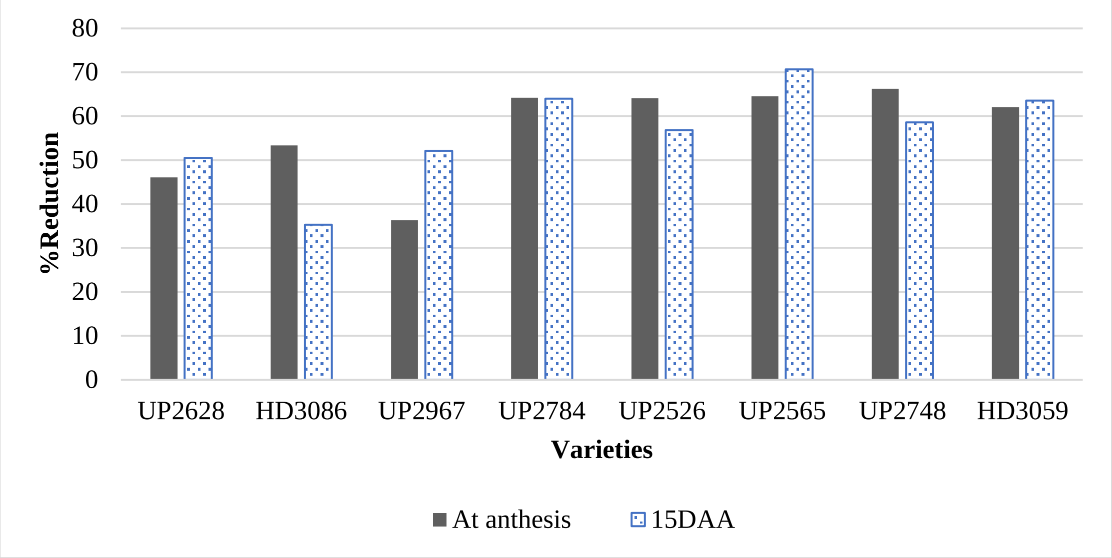


Figure 4.2.2 Reduction in mean values of membrane stability index of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

Membrane injury due to heat stress can be measured by calculating MSI. In the above research, MSI was found to be reduced under D2 in comparison to D1 sowing condition. On comparing between the two sensitive stage, MSI was found higher at the time of anthesis and reduced as the wheat varieties tends towards grain filling. From previous findings, higher temperature stress limits the productivity of winter wheat and the membrane injury can be increased by 6 to 72 % which causes reduction in membrane stability index of crop (MSI) of the crop **(Saadalla *et al.*, 1990).** Through an experiment, similar results were observed in which optimum timing sown wheat varieties showed lower membrane injury (upto 7.48 %) or higher MSI than heat stressed varieties (upto 49.15 %) at both stages (anthesis and 15DAA) (**Chandra, 2006**). An experiment in which different varieties showed different membrane stability under heat stress condition. High MSI was recorded in HD2733, K9006, HP1761, NW1067 and NW1021 under heat stress compared with K8962, NW1076 and NW1041 which showed less MSI in vegetative as well as in reproductive stage (**Singh *et al.*, 2007).** Previous studies also showed that high MSI is positively related to tolerance behaviour of the plant as PBW452×LoK 54 cross was found the most tolerant one by performing least relative injury of 26% as compared to either parent. This study concludes that under high temperature, leakage of ions occurred due to disturbance in cell membrane which is identified by measurement of electrical conductivity and helpful in calculating MSI (**Reddy *et al*., 2008**). The membrane stability index was found lower in heat stress treated PBW343 and C306 genotypes of wheat during anthesis and 15 days after anthesis and a greater reduction was observed in PBW343 (heat sensitive) than in C306 (heat tolerant) (**Almeselmani, 2009**). Similar trend also showed by study in which maximum values of MSI were found for the varieties which are thermo-tolerant i.e. PBW574 (84.82 %), Raj3765 (68.23 %) Lok54 (66.10 %) K0307 (66.09 %) DBW14 (61.18 %) and minimum MSI were found for the varieties which are sensitive towards heat stress i.e. HS240 (30.54 %) and WH1020 (56.79 %) (**Dhyani 2010**). Similarly different wheat genotypes; Halna, PBW 343, NW 1014, DBW 16, K 911 and AAI 11 under different sowing conditions were analysed i.e25 January, and 23 November 2015. The results revealed that the wheat genotypes Halna, NW 1014, DBW 16, K 911, and AAI 11 had high membrane stability index (MSI) with less percent reduction in yield and yield components comparatively to PBW 343 and DBW 16. So, these genotypes with higher MSI can be used as physiologically screened genotypes for heat tolerance because tolerant plant showed less leakage due to accumulation of high saturated fatty acids and monounsaturated fatty (**Jaiswal *et al*., 2017**). Recent findings showed that there is about 2 to 3ºC elevation in average temperature due to elevated temperatures which may have a significant decrease in cell membrane thermo stability index. According to this research MSI of different wheat genotypes under normal sowing were ranged from 29 to 70.6 % while under elevated temperature conditions it become only 9.1 %. Maximum MSI under optimal sowing in genotype SRN-09111 (70.6 %) and Dani (63.7 %) were recorded but under elevated temperature sowing condition they showed 87.11 % relative decrease. The reason for such drastic reduction is due to increase in temperature beyond the threshold level could cause an increase in kinetic energy of molecules across membranes resulting loosening in cell membranes either because of increasing unsaturated fatty acids or due to denaturation of proteins **(Khan *et al*., 2020).** In one study, the reason for high membrane injury under elevated temperatures is that heat stress disturbs the native conformation of membrane proteins that may affect the integrity and function of biological membrane system leading to decrease MSI (**Nijabat *et al*., 2020**).

4.2.3 Canopy Temperature Depression (CTD)

Canopy Temperature Depression (CTD) of different wheat varieties was observed at the time of anthesis and 15 days after anthesis (15 DAA) in two consecutive years 2018-19 and 2019-20. During anthesis, the values of CTD were higher under D1 as compared to D2 (elevated temperatures) condition. That means under D1 the varieties experience cooler canopies as compared to the varieties under D2. Similarly, during grain filling the values of CTD of all wheat varieties were reduced under D2 in comparison to D1 which means that the wheat varieties under D1 are cooler than D2. This is concluding that during both stages the difference between environmental temperature and canopy temperature under D2 is less and the varieties couldn't maintain its cooler canopy under elevated temperatures conditions. Between two sensitive stages cooler canopy (high CTD) observed during anthesis stage as compared to grain filling stage. In detailed view, at anthesis, during 1st trial (2018-19), highest value of CTD was observed in UP2784 variety (8.87) and lowest in HD3059 (7.85) under D1 while under D2, CTD values was found to be reduced and highest value observed in UP2565 variety (8.12) and lowest in HD3086 (6.97). Similarly, during 2nd trial (2019-20), highest CTD was observed in UP2967 variety (8.97) and lowest in HD3086 (7.57) under D1 conditions while under D2, highest CTD was observed in UP2748(7.85) and lowest in UP2967(7.03). On comparing the mean values of two-year trial data (2018-19 and 2019-20), at anthesis maximum value of CTD/cooler canopy was experienced by UP2967 (8.83) and minimum/least cooler canopy by HD3086 (8.03) under D1. However, under D2maximum CTD/cooler canopy was attained for UP2748 variety (7.93) and minimum/least cooler for UP2967 (7.05) (**Table 4.2.3**). Over all at the time of anthesis maximum reduction in CTD values due toD2 (elevated temperatures) was observed in UP2967 (20.19%) while minimum reduction in CTD values was observed in UP2748 (5.65%) (**Figure 4.2.3**). Statistically, the values of CTD under each year, treatment and variety concludes that there was a significant difference in CTD at the time of anthesis between the two-sowing conditions (D1 and D2) ($p \leq 0.01$) and between varieties ($p \leq 0.05$). While there was a non-significant difference was found between the two years ($p > 0.05$). All the other interactions were also found non-significant with each other($p > 0.05$) except for treatment× varieties that was found significant at ($p \leq 0.05$).

At grain filling (15 DAA) during 1st trial (2018-19), highest CTD was observed in UP2967 variety (8.10) and lowest in UP2628 (6.46) under D1 while under D2, CTD values was found to be reduced and highest value observed in UP2967 variety (6.31) and lowest in HD3086 (4.70). Similarly, during 2nd trial (2019-20), highest CTD was observed in HD3059 variety (7.62) and lowest in HD3086 (6.73) under D1 conditions while D2, highest CTD was observed in UP2748 (6.85) and lowest in HD3086 (4.35). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at grain filling maximum CTD/cooler canopy was experienced by UP2967 variety (7.74) and minimum/least cooler canopy by HD3086 (6.65) under D1. However, under D2, maximum CTD/ most cooler canopy was attained for UP2967 and UP2748 variety (6.19) and minimum/least cooler for HD3086 (4.52) (**Table 4.2.3**). Over all at the time of grain filling (15 DAA) maximum reduction in CTD values due to D2 (elevated temperatures) was observed in HD3086 (31.96%) while minimum reduction in CTD values was observed in UP2748 (12.13%) (**Figure 4.2.3**). Statistically, under each year, treatment and variety, there was a significant difference in CTD values at the time of grain filling between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$). While a non-significant difference was found between the two years ($p \leq 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment ×variety and year × variety was found significant at ($p \leq 0.05$) and ($p \leq 0.01$) respectively.

Table 4.2.3 Effect of differential temperatures on the values of Canopy temperature depression (CTD) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

Canopy Temperature Depression												
At anthesis							**15 DAA**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	8.30	8.07	**8.18**	7.05	7.82	**7.44**	6.46	7.43	**6.95**	6.00	6.00	**6.00**
HD3086	8.50	7.57	**8.03**	6.97	7.30	**7.13**	6.56	6.73	**6.65**	4.70	4.35	**4.52**
UP2967	8.70	8.97	**8.83**	7.07	7.03	**7.05**	8.10	7.37	**7.74**	6.31	6.07	**6.19**
UP2784	8.87	8.64	**8.75**	7.33	7.33	**7.33**	7.63	7.40	**7.52**	5.43	5.37	**5.40**
UP2526	8.60	8.00	**8.30**	7.50	7.78	**7.64**	7.53	7.23	**7.38**	5.93	5.69	**5.81**
UP2565	8.70	8.75	**8.73**	8.12	7.47	**7.79**	8.16	7.03	**7.60**	5.50	6.02	**5.76**
UP2748	8.30	8.50	**8.40**	8.00	7.85	**7.93**	6.53	7.57	**7.05**	5.53	6.85	**6.19**
HD3059	7.85	8.27	**8.06**	7.53	7.43	**7.48**	7.79	7.62	**7.70**	5.53	5.01	**5.27**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.065		0.130		0.185		0.093		0.186		0.264	
CD (5%)	0.185		0.370		0.523		0.264		0.528		0.747	

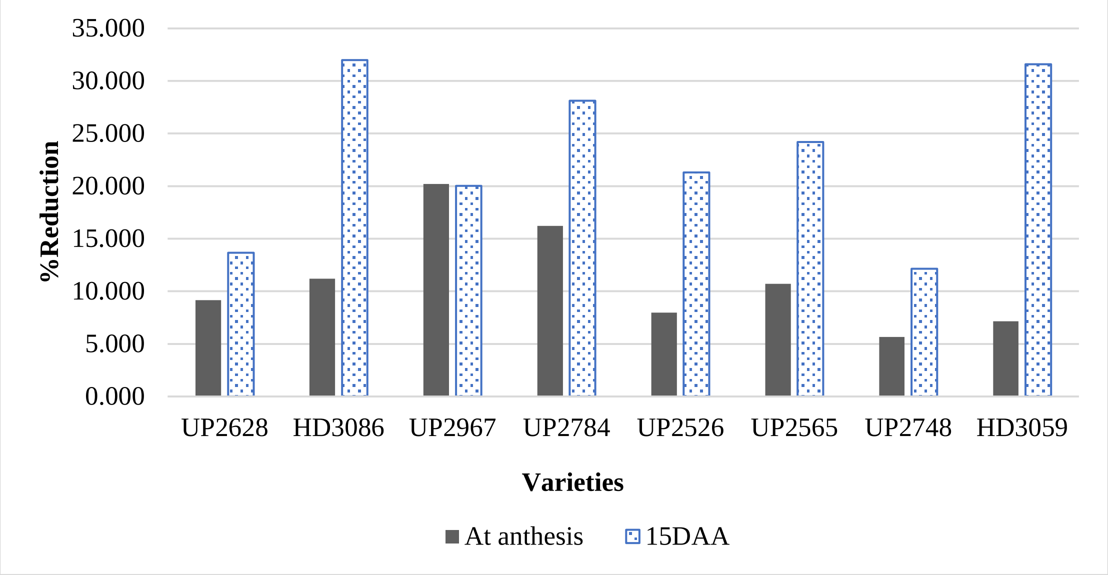


Figure 4.2.3 Percent reduction in mean values of Canopy temperature depression (CTD) of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present research during anthesis, the canopy temperature depression CTD values was found higher under D1 as compared to D2 which conclude that under D1 condition, canopy temperature was found less (cooler canopy) in comparison to the canopy temperature of wheat varieties which are grown under D2. According to previous research, higher CTD a for variety means it retained cooler canopies than the varieties having lower CTD value. Mostly heat tolerant wheat varieties (Gouran, Sourav, Kanchan and Shatabdi) exhibited higher CTD than heat sensitive lines (Sonora and Kalyansona) (**Sikder and Paul, 2010**). Similar saying also observed by other researchers (**Saxena *et al*., 2014**). However, comparing the wheat varieties on the bases of CTD values in their sensitive stages (anthesis and 15 DAA/grain filling), the CTD values found lower during grain filling under both sowing conditions as compared to anthesis stages. From previous study it is reported that lower canopy temperature during grain filling period is an important physiological principle for high temperature stress tolerance in various crop species such as cotton, wheat etc. but if canopy temperature was found higher during grain filling that means at that stage plant can’t maintain the cooler canopies and this will affect the filling of the grains and ultimately the grain weight (**Mason and Singh, 2014**). Studies also conclude that Canopy temperature depression (CTD) is frequently used as an essential criterion to find out the degree to which particular variety canopy is cooler than its surrounding environment. As in an experiment in different wheat varieties there was no significant difference in CTD between 23 Oct and 6 Nov, but there was high significant difference in CTD under 22 Nov. and 06 Dec. treatments during anthesis and milky grain stages (grain filing stage). The reason for such significant differences is that transpiration causes canopy temperature to drop below the air temperature but under warm and sunny weather conditions decrement in canopy temperature depression observed (**Nazeri, 2017**). Similar results also observed in some other researches in which all the wheat genotypes maintain cooler canopy under normal growing condition as compared to both moderate and high temperature sowing conditions. After 18 days of anthesis (grain filling stage) similar trend of decreasing CTD under elevated temperatures as compared to non-stress sowing conditions was also observed. Higher CTD under high temperature growing condition (BARI Gom-26 and BAW-1202) comparatively than other wheat genotypes (BAW-1182 and BARI Gom-27) are considered as temperature tolerant genotype. Under heat stress, differences in the performance of different wheat genotype are may be the increased respiration and transpiration (as a result of stomatal closure) of superior tolerant genotypes that performs higher canopy depression under heat stress condition along with they could have increased stay green duration with high chlorophyll content that enhance photosynthetic activity of tolerant genotypes (**Sharmin *et al*., 2020**).

4.2.4 Chlorophyll Fluorescence (Fv/Fmax)

Chlorophyll Fluorescence (Fv/Fmax) of different wheat varieties was observed at the time of anthesis and 15 days after anthesis (15 DAA) in two consecutive years 2018-19 and 2019-20. In all wheat varieties, the values of Fv/Fmax were reduced under D2 (elevated temperatures) in both years at anthesis as well as 15 DAA (grain filling) as compared to the values under D1. On comparing between the stages, chlorophyll fluorescence in all wheat varieties was found higher at the time of anthesis as compared to grain filling stage (15 DAA) which means that when the varieties tend towards grain filling, chlorophyll fluorescence was found reduced under both sowing conditions (D1 and D2). At anthesis, during 1st trial (2018-19), highest value of Fv/Fmax was observed in UP2748 variety (0.78) and lowest in HD3086 (0.71) under D1 while under D2, the Fv/Fmax values was found to be reduced and highest value was observed in UP2748 variety (0.76) and lowest in UP2784 (0.68). Similarly, during 2nd trial (2019-20), highest Fv/Fmax was observed in UP2967 variety (0.80) and lowest in HD3086(0.72) under D1 while under D2, highest Fv/Fmax was observed in UP2748(0.76) and lowest in UP2784 (0.67). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at anthesis maximum value of chlorophyll fluorescence was attained by UP2967 and UP2748 variety (0.78) and minimum by HD3086 (0.72) under D1. However, under D2, maximum value of chlorophyll fluorescence was attained for UP2748 variety (0.76) and minimum for HD3086 (0.67) (**Table 4.2.4**). Over all at the time of anthesis maximum reduction in chlorophyll fluorescence due to D2 (elevated temperatures) was observed in UP2967 (8.30 %) while minimum reduction in chlorophyll fluorescence was observed in UP2565 (2.24 %) (**Figure 4.2.4**). Statistically, under each year, treatment and variety concludes that there was a significant difference in fv/fmax values at the time of anthesis between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were found non-significant with each other ($p \leq 0.01$).

At grain filling (15 DAA) during 1st trial (2018-19), highest value of Fv/Fmax was observed in UP2748 variety (0.71) and lowest in UP2784 (0.63) under D1 while under late sowing, the Fv/Fmax values was found to be reduced and highest value was observed in UP2748 variety (0.64) and lowest in UP2628 (0.57). Similarly, during 2nd trial (2019-20), highest Fv/Fmax was observed in UP2748 variety (0.72) and lowest in UP2628 and UP2526 (0.66) under D1 conditions while under D2, highest Fv/Fmax was observed in UP2565 (0.67) and lowest in HD3059(0.54). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that at grain filling maximum value of chlorophyll fluorescence was attained by UP2565 and UP2748 variety (0.71) and minimum by UP2784 (0.63) under D1. However, under D2, maximum value of chlorophyll fluorescence was attained for UP2565 variety (0.65) and minimum for HD3059 (0.56) (**Table 4.2.4**). Over all at the time of grain filling maximum reduction in chlorophyll fluorescence due to D2 (elevated temperatures) was observed in HD3059 (16.58%) while minimum reduction in chlorophyll fluorescence was observed in UP2784 (2.89%) (**Figure 4.2.4**).

Statistically, under each year, treatment and variety concludes that there was a significant difference in Fv/Fmax values at the time of grain filling between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$)

Table 4.2.4 Effect of differential temperatures, on the values of chlorophyll fluorescence (Fv/Fmax) of different wheat varieties at anthesis and 15DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

Chlorophyll Fluorescence												
At anthesis							15 DAA					
Varieties	**D1**			**D2D**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	0.76	0.77	**0.77**	0.75	0.73	**0.74**	0.66	0.66	**0.66**	0.57	0.56	**0.57**
HD3086	0.71	0.72	**0.72**	0.70	0.69	**0.70**	0.69	0.67	**0.68**	0.61	0.61	**0.61**
UP2967	0.77	0.80	**0.78**	0.71	0.73	**0.72**	0.70	0.70	**0.70**	0.62	0.62	**0.62**
UP2784	0.72	0.73	**0.73**	0.68	0.67	**0.67**	0.63	0.64	**0.63**	0.60	0.63	**0.62**
UP2526	0.74	0.74	**0.74**	0.72	0.73	**0.73**	0.64	0.66	**0.65**	0.63	0.62	**0.63**
UP2565	0.74	0.76	**0.75**	0.73	0.74	**0.73**	0.70	0.71	**0.71**	0.63	0.67	**0.65**
UP2748	0.78	0.78	**0.78**	0.76	0.76	**0.76**	0.71	0.72	**0.71**	0.64	0.65	**0.64**
HD3059	0.75	0.76	**0.76**	0.74	0.73	**0.73**	0.65	0.69	**0.67**	0.58	0.54	**0.56**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.004		0.008		0.012		0.006		0.012		0.017	
CD (5%)	0.012		0.025		0.035		0.017		0.035		0.050	

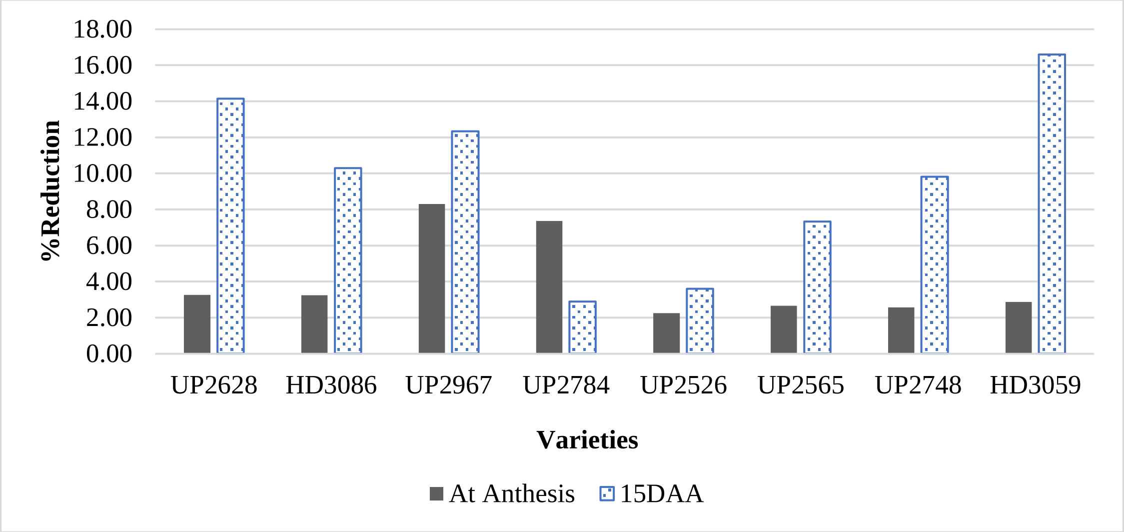


Figure 4.2.4 Reduction in mean values of chlorophyll fluorescence (Fv/Fmax) of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In investigated research, chlorophyll fluorescence (Fv/Fmax) was found to be reduced under elevated temperature (D2) as compared to D1 sown condition in both years under both sensitive stages (anthesis and grain filling). The reason for such decrement according to previous reports, is that under higher temperature (heat stress) reduction in photosynthetic efficiency occurs as a result of breakdown of proteins (PSII and Rubisco) and by destruction of membranes by lipid degradation leads to decrease in chlorophyll fluorescence values (**Hassan, 2006**). Heat stress also damages PSII active site, separate LHCII from PSII core complex as a results inhibition of PSII electron transport occurs hence photosynthetic inhibition takes place (**Ristic *et al*., 2007**). On comparing the present investigated results of chlorophyll fluorescence between the two sensitive stages, chlorophyll fluorescence was found higher during anthesis as compared to 15 DAA under both sowing conditions. Similar results also expressed by some experiment in which Fv/Fmax ratio of flag leaves in wheat decreased after 14 days of anthesis (**Cai *et al*., 2008**). The reason for such stage specific decrement is that as photosynthesis depends on the function of light harvesting and electron transport system within chloroplast (expressed as photochemical efficiency) and measured by chlorophyll a fluorescence variable yield (Fv/Fmax) ratio, due to elevation in temperature during grain filling more than the elevation in temperature during anthesis stage leads to destruction in light harvesting complexes and proteins associated with it causes decrement in photosynthesis efficiency/chlorophyll fluorescence **(Prasad *et al.*, 2008a)**. Similar results also observed in other experiments in which effect of heat stress at 37 °C and 45 ºC for 8 hours on seedling of Karacadag and Firat wheat cultivars was studies and it was found that the chlorophyll florescence was significantly decreased in heat treatment (45 ºC) (**Efeoglu and Terzioglu, 2009).** Previous reports also suggest that PSII is found more sensitive to high temperature as under heat stress thylakoid membrane fluidity and electron transport is directly dependent on PSII integrity and damage to PSII leads to reduced carbon fixation, oxygen evolution and disturbs linear flow of electrons in thylakoid membrane This is the most important reason for reduced photosynthetic efficiency under elevated temperatures (**Farooq *et al*., 2011).** According to a study, in which six wheat cultivars (UP-2338, PBW-343, UP-2113, PBW-175, VL-616 and

VL-421) were subjected to variable temperature conditions by sowing them in two different dates with a delay 20 days. Chlorophyll fluorescence variable yield (Fv/Fm), which indicates the functionality of PSII got impaired under late sowing. The higher Fv/Fm values just after anthesis shows better performance of PSII, which starts getting down regulated as the varieties tends towards maturity. The results concludes that under late sowing, photosynthetic efficiency gets adversely affected. This trend was quite similar in all the cultivars (**Srivastava *et al.*, 2012**). In another field experiment, 30 wheat genotypes were analysed for PSII thermostability by using the chlorophyll fluorescence kinetics. Basal Chlorophyll fluorescence increased above 44 ºC in all wheat genotypes indication PSII damage due to temperature rise (**Brestic *et al.*, 2013**). In another study, seven winter wheat varieties were exposed to two treatments in a growth chamber [35/15 °C (heat stress) and 25/15 °C (control) day/night] and in field-condition heat tents (imposed +6 °C higher than ambient). The results showed there was decline in QY, particularly in wheat cultivars Larry and WB4458 (susceptible towards heats stress) **(Šebela *et al.*, 2020).** In a recent research, four wheat genotypes heat-sensitive: LM19, SF1 and heat-tolerant: LM62, NS3 were treated with heat treatment (36/26 °C Day/Night temperature) and combined drought+heat stress (36/26 °C Day/Night temperature with 40/60 % Day/Night). The results showed the general trend in which significant decrement with heat stress in the values of Fv/Fm was observed. Higher Fv/Fman values was observed in heat-tolerant compared to the heat-sensitive genotypes under both heat and drought+heat treatment **(Abdelhakim *et al.*, 2021).**

Correlation analysis between physiological parameters with grain yield under both sowing conditions.

By taking the average of two years data, correlation was done for physiological parameters with grain yield under D1 and D2. No significant correlation was observed between grain yield and other physiological parameters under D1, however positive correlation was observed for grain yield with Relative water content (RWC), Membrane stability Index (MSI), and Canopy temperature depression (CTD) but the correlation was non-significant. On comparing between other physiological parameters non-significant positive correlation observed between RWC & MSI, MSI

& CTD, Chlorophyll fluorescence (CF) & CTD while significant negative correlation was observed between RWC and CF (r=-0.75*). Non-significant negative correlation was also observed under D1 in between RWC & CF, RWC & CTD, MSI & CF. Under D2, grain yield found positively correlated with MSI (r=0.65) CF and CTD but the values were non-significant or very less. On comparing between other physiological parameters positive correlation was observed between RWC & MSI (r=0.45) and CF and CTD (r=0.64). While rest of the physiological parameters were found negatively correlated with each other. From this it can be concluded that sowing conditions (in November or December) does not show any significant difference between the relationships of their physiological parameters. However, more negative and significant correlation was observed between physiological parameters for D2 (December sown) that could also be as reason of reduced grain yield under D2in comparison to D1 (**Table 4.2.5.1 and Table 4.2.5.2).**

Table 4.2.5.1 Pearson's correlation between physiological parameters with grain yield under D1.

	GY	*RWC*	*MSI*	*CF*	*CTD*
GY	1				
RWC	0.02	1			
MSI	0.18	0.35	1		
CF	-0.37	-0.75*	-0.49	1	
CTD	0.01	-0.40	0.24	0.26	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Relative water content (RWC), Membrane stability Index (MSI), Chlorophyll fluorescence (CF)and Canopy temperature depression (CTD).

Table 4.2.5.2 Pearson's correlation between physiological parameters with grain yield under D2.

	GY	*RWC*	*MSI*	*CF*	*CTD*
GY	1				
RWC	-0.01	1			
MSI	0.65	0.45	1		
CF	0.12	-0.13	-0.24	1	
CTD	0.08	-0.46	-0.56	0.64	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Relative water content (RWC), Membrane stability Index (MSI), Chlorophyll fluorescence (CF)andCanopy temperature depression (CTD).

4.3 Effect of elevated temperatures on yield and yield attributes

4.3.1 Spike length (cms.) and Spike weight (grams)

The spike length and spike weight of each wheat variety was observed at the time of maturity, in two consecutive years 2018-19 and 2019-20. In all wheat varieties, spike length and spike weight were found to be reduced due to D2 (elevated temperatures) in both the years as compared to D1sown conditions. For spike length, during 1st trial (2018-19), highest spike length was observed in UP2565 variety (13.44 cm) and lowest in UP2526 (9.17 cm) under D1 while under D2, spike length was found to be reduced and highest value observed in UP2565 (11.30 cm) and lowest in HD3059 variety (8.39 cm). Similarly, during 2nd trial (2019-20), highest spike length observed in UP2565 (12.33 cm) and lowest in HD3086 (8.89 cm) under D1 while under D2, highest spike length was observed in UP2565 (11.90 cm) and lowest in HD3086 (6.64 cm). On comparing the mean values of two-year trial data (2018-19 and 2019-20), maximum spike length was attained by UP2565 variety (12.89 cm) and minimum by UP2526 (9.08 cm) under D1 sown conditions. However, under D2, maximum spike length was attained byUP2565 variety (11.60 cm) and minimum byHD3059 (8.04 cm) (**Table 4.3.1).** Over all maximum reduction in spike length due to D2 (elevated temperatures) was recorded in HD3059 (19.15 %) while minimum in UP2748 (5.91 %) (**Figure 4.3.1**). Statistically spike length was significantly affected by D2 in terms of years, treatments and varieties ($p \leq 0.01$). All the other interaction were found non-significant except interactions between years × treatment which was found significant at ($p \leq 0.05$).

During 1st trial (2018-19), highest spike weight was observed in variety UP2967 (3.70 grams) and lowest in UP2628 (2.09 grams) under D1 sown condition while under D2, spike weight was found to be reduced and highest value observed in HD3059 (2.96 grams) and lowest in UP2628 (1.62 grams). Similarly, during 2nd trial (2019-20), highest spike weight was observed in UP2967 (3.61 grams) and lowest in UP2628 (2.23 grams) under D1 while under D2, highest spike weight observed in UP2967 (2.97 grams) and lowest in UP2628 (1.65 grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum spike weight was attained by UP2967 (3.66 grams) and minimum by UP2628 (2.16 grams) under D1.

However, under D2, maximum spike weight was attained by UP2967 variety (2.87 grams) and minimum by UP2628 (1.63 grams) (**Table 4.3.1**). Over all maximum reduction in spike weight due to D2 (elevated temperatures) was recorded in UP2784 variety (37.44 %) while minimum in UP2748 and HD3059 (8.66 % and 5.64 % respectively) (**Figure 4.3.1**). Statistically spike weight under each year, treatment and variety concludes that there was a significant difference in spike weight at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$).

Table 4.3.1 Effect of differential temperatures on spike length (cm) and spike weight (grams) of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

Varieties	Spike length (cm)						Spike weight (grams)					
	D1			D2			D1			D2		
	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean	2018-19	2019-20	Mean
UP2628	11.11	10.00	**10.56**	9.48	8.33	**8.91**	2.09	2.23	**2.16**	1.62	1.65	**1.63**
HD3086	9.94	8.89	**9.42**	9.51	6.64	**8.07**	3.33	3.35	**3.34**	2.23	2.26	**2.25**
UP2967	11.22	10.28	**10.75**	9.92	8.17	**9.05**	3.70	3.61	**3.66**	2.78	2.97	**2.87**
UP2784	11.33	11.11	**11.22**	10.18	9.33	**9.76**	3.44	3.34	**3.39**	2.14	2.10	**2.12**
UP2526	9.17	9.00	**9.08**	8.63	7.11	**7.87**	3.68	3.38	**3.53**	2.72	2.70	**2.71**
UP2565	13.44	12.33	**12.89**	11.30	11.90	**11.60**	3.64	3.13	**3.39**	2.62	2.62	**2.62**
UP2748	9.44	9.16	**9.30**	9.42	8.08	**8.75**	2.74	2.78	**2.76**	2.54	2.50	**2.52**
HD3059	9.89	10.01	**9.95**	8.39	7.70	**8.04**	2.98	2.91	**2.95**	2.96	2.60	**2.78**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.116		0.232		0.328		0.068		0.136		0.193	
CD (5%)	0.328		0.657		0.929		0.192		0.385		0.545	

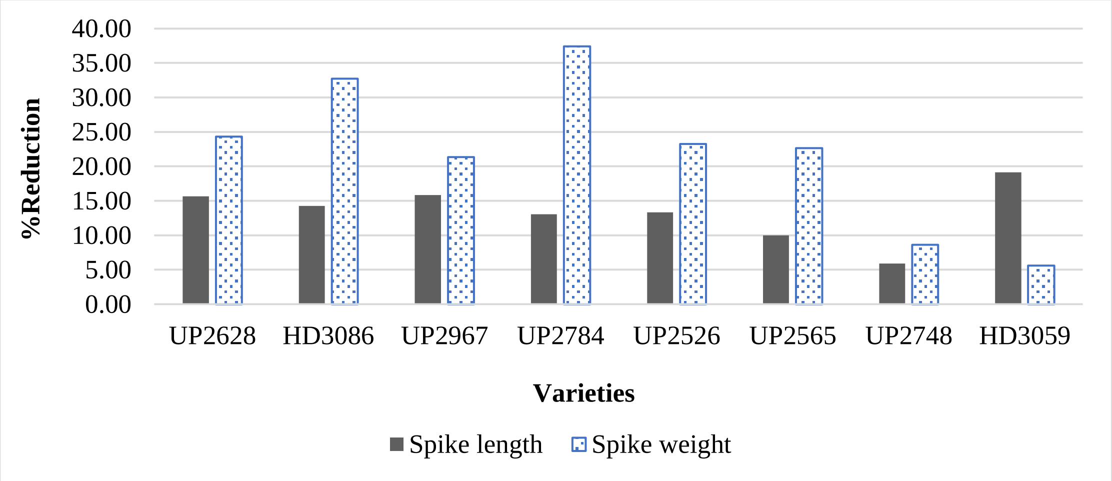


Figure 4.3.1 Reduction in mean values of spike length and weight of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.3.2 Spike number and total spike weight per plant

The spike number and total spike weight per plant of each wheat variety was observed at the time of maturity, in two consecutive years 2018-19 and 2019-20. In all wheat varieties, spike number and spike weight per plant were found reduced under D2 (elevated temperatures) conditions in both the years as compared to D1 sown conditions. For spike number per plant, during 1st trial (2018-19), highest spikes per plant was observed in UP2784 variety (9.67) and lowest in HD3059 (5.00) under D1 while under D2, spikes per plant was found to be reduced and highest value observed in UP2784 variety (6.11) and lowest value in HD3059 (4.33). Similarly, during 2nd trial (2019-20), highest spike number per plant were observed in UP2784 variety (9.00) and lowest in HD3059 (5.78) under D1 while under D2, highest number of spikes observed in UP2784(6.78) and lowest in UP2967 (4.00). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum spikes/plant was attained by UP2784 variety (9.33) and minimum by HD3059 (5.39) under D1. However, under D2, maximum spikes/plant was attained byUP2784 variety (6.44) and minimum by HD3059 (4.28) (**Table 4.3.2).** Over all maximum reduction in spikes/plant due to D2 (elevated temperatures) was recorded in UP2967 (44.53 %) while minimum reduction in spikes/plant was observed in HD3086 (14.53 %) (**Figure 4.3.2**).Statistically, spikes/plant under each year, treatment and variety concludes that there was a significant difference in spike number/plant at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant (($p \leq 0.05$).

For total spikes weight per plant, during 1st trial (2018-19), highest weight of the total spikes per plant was observed in UP2967 (14.23 grams) and lowest in HD3059 (8.23 grams) under D1 while under D2, total spikes weight per plant was found to be reduced and highest value observed UP2628 variety (9.16 grams) and lowest in HD3059 (5.73 grams). Similarly, during 2nd trial (2019-20), highest weight of spikes per plant was observed in UP2967 variety (14.23 grams) and lowest in

UP2748 (8.76 grams) under D1 while under D2, highest weight of spikes was observed in UP2967 (8.78 grams) and lowest in HD3059 (5.57 grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum spikes weight/plant was attained by UP2967 variety (14.23 grams) and minimum by HD3059 (8.53 grams) under D1. However, under D2, maximum spikes weight/plant was attained by UP2967 variety (8.96 grams) and minimum by UP2748 (5.65) (**Table 4.3.2).** Over all maximum reduction in total spike weight/plant due to D2 (elevated temperatures) was recorded in UP2526 (44.52 %) while minimum reduction in total spike weight/plant was observed in UP2748 (4.51 %) (**Figure 4.3.2**). Statistically total spike weight/plant under each year, treatment and variety concludes that there was a significant difference at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant (($p \leq 0.05$).

Table 4.3.2 Effect of differential temperatures on number of total spike and total spikes weight (grams) per plant of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

	Number of spikes per plant						**Total spike weight per plant (grams)**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	6.11	6.00	**6.06**	4.56	5.33	**4.94**	9.25	9.68	**9.46**	9.16	8.02	**8.59**
HD3086	6.33	6.67	**6.50**	5.56	5.56	**5.56**	9.17	9.43	**9.30**	7.61	7.15	**7.38**
UP2967	7.67	7.56	**7.61**	4.44	4.00	**4.22**	14.23	14.23	**14.23**	9.13	8.78	**8.96**
UP2784	9.67	9.00	**9.33**	6.11	6.78	**6.44**	13.60	13.03	**13.32**	9.13	8.53	**8.83**
UP2526	7.00	7.56	**7.28**	5.00	5.22	**5.11**	13.43	13.84	**13.63**	8.51	6.62	**7.57**
UP2565	7.67	7.78	**7.72**	4.67	4.33	**4.50**	12.78	12.77	**12.78**	8.80	8.21	**8.51**
UP2748	6.83	6.78	**6.81**	5.56	5.33	**5.44**	8.62	8.76	**8.69**	7.83	8.77	**8.30**
HD3059	5.00	5.78	**5.39**	4.33	4.22	**4.28**	8.23	8.82	**8.53**	5.73	5.57	**5.65**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.133		0.267		0.377		0.198		0.397		0.562	
CD (5%)	0.377		0.755		1.068		0.561		1.123		1.589	

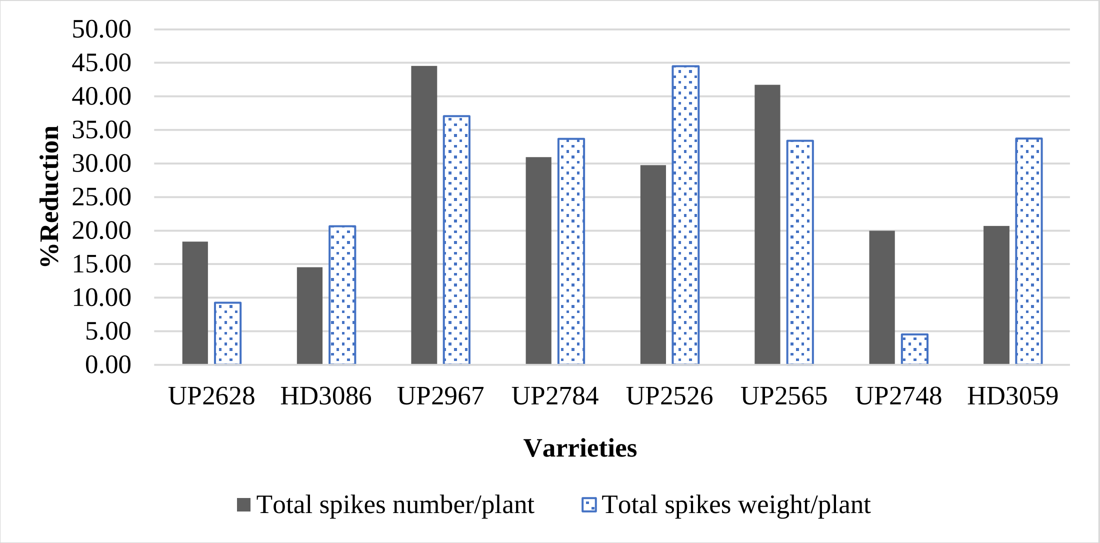


Figure 4.3.2 Reduction in mean values of total spikes number and weight per plant of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

Under D2 (elevated temperature conditions) all the spike characteristics (as mentioned above) were found reduced in comparison to D1 (non-stressed conditions. Previous researches also followed the similar trend in which various spike characters as length, weight and number were found reduced under heat stress condition as compared to controlled/non-heat stressed condition. An experiment was conducted in different wheat varieties namely HD2687, UP2113, VL616, PBW343, UP2338, PBW175, UP2425 and VL421 under two sowing dates i.e 27th November and 20th December. Spike length was found to be longer in case of November sown varieties as compared to the same varieties when sown in December month. A reduction range 7.40 % to 12.90 % was reported in spike length in different varieties under elevated temperature conditions in comparison to normal conditions. The reduction may be due to high temperature which hastened spike initiation, its growth and development by shortening the vegetative growth of plant and acceleration towards maturity causes reduction in various spike characters **(Srivastava, 2005).** Similar results in which length of the spikes was found longer in case of wheat cultivar sown on 25th November and 23rd November as compared to wheat varieties sown on 19th December and 17th December. Minimum reduction (from 6.11 % to 6.37 %) in spike length was observed for wheat genotypes VL738, Raj3765, PBW396 while other genotypes of wheat showed increased reduction in spike length (from 7.825 to 10.46 %) for PBW373, UP2382, VL802, C306, UP1109. Warmer temperature under delay in sowing conditions causes inappropriate growth and development of plants parts which are directly related to spike formation and grain filling resulting reduced spike length, number and weight under heat stress condition (**Chandra, 2006).** Other studies also justify those higher temperatures (elevation in temperatures) due to inappropriate sowing timing, affect all phases of crop growth, accelerate floral initiation, reduce the period of spike development, resulting in shorter spike with lower number of spikelets, bad pollen development and adversely affects weight of the spikes per plant (**Wahid *et al*. 2007**). In another research, spike length and spike weight found adversely affected by sowing dates, as 20 November sown wheat genotypes had relatively longer length in comparison to varieties sown at 23 December. Maximum reduction in spike length was observed for genotypes HD2733 (39.13%) and minimum for Raj4101 (2.11%) while maximum reduction in spike weight was observed for genotypes 34WH1022 (32.59%) and minimum for 29PBW575 (2.48%) **(Dhyani, 2010).** In an investigation, high temperature at the time of grain filling was continued until ripening and showed a negative impact on yield and yield related measures like kernel weight which can reduce up to 20.61% due to 1–2-degree elevation in temperature hence, this could be a reason for reduced spike weight and total spike weight per plant under elevated temperatures in wheat. Higher temperatures also further associate with limitation of water that cause rapid shrinkage of grain volume hence reduced kernel weight and ultimately the spike weight of the wheat crop (**Modarresi *et al*., 2009**). It was also found that heat stress impairs the viability of anther and pollen, resulting in poor fertilization. The sensitivity of pollen towards high temperature is due to incapability to produce HSPs in response to heat stress. Elevated temperature during meiosis and growth of ovaries could also be as reason for reduction in grain weight in wheat crop under delayed sowing hence could be the reason for reduced spike weight under heat stress condition (**Farooq *et al*., 2011**). Plants have limited nutrient uptake capacity and photosynthetic efficiency under heat stress which can also reduce organ size (leaf, tiller, and spikes (**Ihsan *et al*., 2016**). Heat stress also found responsible for disturbance in kernel morphology by compacting the duration of grain filling period. Due to post-anthesis heat stress, parameters such as grain filling duration and grain weight per spike (finally total spike weights in a plant) were found reduced in comparison to control (no stress condition) (**Iqbal *et al.*, 2017**). A recent study with 18 wheat genotypes showed that 8 % reduction in number of spike/m^2 and 13.50%in peduncle length under late plantation on March 1, 2016 as compared to optimal planting condition (October 1, 2015) while in spike length there was 4.2 % increment due to late planting (**Youldash *et al.*, 2020).** Increased stress in wheat significantly reduces the crop yield by influencing allometric and yield contributing traits. Spike length of wheat seeds variety Faisalabad-2008, was found (14.03 cm) under no stress/controlled condition (well-watered while under heat stress (inside the plastic tunnel) it was reduced to (8.72 cm) (**Sattar *et al.*, 2020**). Spike weight also found reduced by 22.7 % under heat stress condition (December sown) as compared to normal condition (mid-November) in 24 different wheat genotypes (**Kumar *et al.*, 2020).** In contrast 58 genotypes of wheat were observed under two different sowing condition i.e. under stress (high temperature, sowing date - March 1) and optimal (sowing date - October 1). Increment in spike length (4.2 %) and reduction in number of spikes per m^2 (8.00 %) was observed under stress condition as compared to optimal condition. However, the increment in spike length under elevated temperatures in comparison to normal sowing was found non-significant (**Youldash *et al.*, 2020**). The performance of agronomical characteristics of wheat germplasm with different yield-related trait was evaluated under heat stress treatment of 35- 40°C (with the help of glass chamber) verses normal condition. The result showed that extreme temperature causes reduction to grain yield and yield components such as spike length and spike weight. On an average, spike length was found to reduced upto 23.52 % in some of the wheat genotypes while spike weight ranges from 2.003 to 2.920 grams was found under normal temperature and under high temperature, it was reduced and become 1.333 to 2.003 grams. The reduction under elevated temperature may be due to shortening of growth phase and photosynthetic duration in plants under stress condition which results in less deposition of starch by injury of the starch synthesizing enzyme in them (**Khan *et al.*, 2020**). In a recent study, two spring wheat cultivars (Millet-11, Punjab-

11) & (V-07096, V-10110) were investigated in response to terminal heat stress (due to delayed sowing condition). Based on their morpho-physiological traits, the results concludes that elevation in temperature due to inappropriate sowing timing causes reduction in spike length (13 %) under heat stress conditions in comparison to normal condition **(Tariq *et al*., 2021).**

4.3.3 Total spikelets per spike and Grain number per spike

The total spikelets per spike and grains per spike of different wheat varieties were recorded under both sowing condition in two consecutive years 2018-19 and 2019-20 at maturity and the results showed reduction in total spikelets per spike and grains per spike under D2 in both years as compared to D1. In detailed view, during 1st trial (2018-19), highest number of spikelets/spikes were observed in variety UP2967 (88.11) and lowest in UP2748 (65.00) under D1 while under D2, highest spikelets/spike observed in variety UP2784 (70.33) and lowest in UP2565 (54.33). Similarly, during 2nd trial (2019-20), highest spikelets/spikes was observed in UP2967 variety (89.00) and lowest in HD3059 (64.00) under D1 while under D2, highest spikelets/spike observed in UP2784 (69.67) and lowest in HD3086 (55.00). On comparing the mean values of two-year trial data (2018-19 and 2019-20), maximum spikelets/spike was gained by UP2967 variety (88.56) and minimum by UP2748 (64.89) under D1. However, under D2, maximum spikelets/spike was gained for UP2784 variety (70.00) and minimum for UP2565 (55.00) (**Table 4.3.3).** Over all maximum reduction in spikelets/spike due to D2 (elevation in temperature) was recorded in HD3086 (30.89%) while minimum in UP2628 and HD3059 (5.10 % and 6.16 %) respectively (**Figure 4.3.3**). Statistically, a significant difference in spikelets/spike at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant (($p \leq 0.05$).

During 1st trial (2018-19), highest grains/spike was observed in UP2565 (57.00) and lowest in UP2748 (40.22) under D1 while under D2, grains/spike was found to be reduced and highest value was observed in UP2967 variety (54.00) and lowest in UP2628 (32.22). Similarly, during 2nd trial (2019-20), highest grains/spikes was observed in UP2565 variety (58.67) and lowest in UP2748 (37.22) under D1 while under D2, highest grains/spike was observed in UP2967 (57.33) and lowest in UP2748 (33.78). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum grains/spike was recorded in UP2565 variety (57.83) and minimum by UP2748 (38.72) under D1. However, under D2 maximum grains/spike was recorded in UP2967 variety (55.67) and minimum for UP2628 (33.39) (**Table 4.3.3).** Over all maximum reduction in grains/spike due to D2 (elevated temperatures) was recorded in UP2628 (26.44 %) while minimum reduction in grains/spike was observed in UP2967 and UP2526 (2.53 % and 3.78 % respectively) (**Figure 4.3.3**). Statistically a significant difference in grains/spike at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety ($p \leq 0.01$) and year × variety ($p \leq 0.05$) that was found significant.

Table 4.3.3 Effect of differential temperatures on total spikelets per spike and Grains per spike of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

	Spikelets per spike						**Grains per spike**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	68.67	66.33	**67.50**	63.44	64.67	**64.06**	45.11	45.67	**45.39**	32.22	34.56	**33.39**
HD3086	80.00	83.67	**81.83**	58.11	55.00	**56.56**	44.78	44.56	**44.67**	39.67	35.33	**37.50**
UP2967	88.11	89.00	**88.56**	69.33	65.00	**67.17**	55.89	58.33	**57.11**	54.00	57.33	**55.67**
UP2784	77.44	75.67	**76.56**	70.33	69.67	**70.00**	50.78	43.22	**47.00**	47.22	40.67	**47.44**
UP2526	73.89	70.33	**72.11**	62.56	55.33	**58.94**	49.22	44.89	**47.06**	48.11	42.44	**45.28**
UP2565	77.67	77.33	**77.50**	54.33	55.67	**55.00**	57.00	58.67	**57.83**	43.56	45.67	**44.61**
UP2748	65.00	67.67	**66.33**	55.67	54.44	**55.06**	40.22	37.22	**38.72**	37.33	33.78	**35.56**
HD3059	65.78	64.00	**64.89**	60.67	61.11	**60.89**	43.33	43.11	**43.22**	35.00	34.33	**34.67**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.655		1.310		1.853		0.601		1.203		1.701	
CD (5%)	1.852		3.704		5.239		1.701		3.402		4.811	

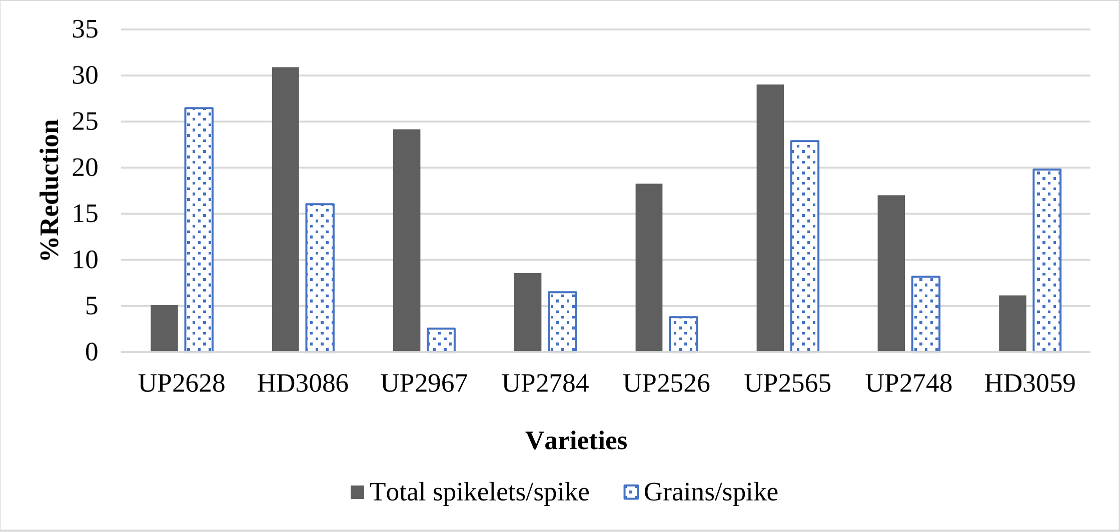


Figure 4.3.3 Reduction in mean values of total spikelets/ spike and grains/spike of different varieties under D2as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

The present results showed that under D2 sown conditions, spikelets per spike and grains per spike were found reduced in comparison to the wheat varieties which were sown under D1 conditions. As spikelet number and grain number are influenced by other phenological stage during plant development such as spikelet initiation, floral organ differentiation, male female sporogenesis, pollination and fertilization. Under heat stress sudden speeding up of these developments causes improper growth hence reduces spikelet number and number of grains per spike in wheat (**Saini and Aspinall, 1982, Porter and Gawith, 1999**). The trend of reduction in spikelets number and grain number under elevated temperatures also followed by previous researches. An experiment was conducted with 14 spring wheat genotypes, which were exposed to heat stresses from 30 to 38ºC which cause reduction in grain number by 20 to 44%. This reduction is may be due to improper growth of spike and improper differentiation of spikelets under elevated temperatures (**Tahir and Nakata, 2005**). In an experiment number of grains per spike was found reduced under wheat varieties sown on 19th December and 17th December when compared to wheat cultivar sown on 25th November and 23rd November. A reduction from 2.5 % to 31.34 % in grains/spike was observed in all wheat varieties. The reason for such reduction maybe due to warmer temperature which causes inappropriate growth, development and grain filling and results reduction in number of grains per spike (**Chandra, 2006).** High temperature

>30ºC during floret development and floret initiation in wheat life cycle could cause reduction in grain number or sometimes complete sterility in wheat (**Anjum *et al.*, 2008**). Elevated temperature also causes shrinking of grains through changes in ultra-structure inside aleurone layer and endosperm cells of kernel when day night temperature increased from 25/14ºC to 31/20ºC. The reason for such changes was found as in absence of heat stress wheat grains has larger cells surrounded by starchy endosperm but under heat stress, the endosperm cellular structure is denser, packed with starch granules embedded in protein matrix **(Dias *et al.*, 2008**). Higher temperatures affect all phases of crop growth, accelerate floral initiation, reduce the period of spike development, resulting in shorter spike with lower number of spikelets, and adversely affecting pollen development. A study in which yield and yield related traits of 144 recombinant inbred lines (RILs) of wheat derived from the cross of Kauz (heat tolerant) and MTRWA116 (susceptible) together under normal and heat stress conditions were analysed. The results showed a significant difference among RILs for all traits in stress and control conditions. High temperature significantly decreased all the yield related traits such as spikelets per spike by 11.77%, grains per spike was affected more by high temperature (23.61%). The decrease in number of kernels per spike was most probably due to increased sterile spikelets **(Modarresi *et al.*, 2009).** In another research, grains per spike of wheat genotypes were found reduced under elevated temperatures (23 December) in comparison to varieties sown on 20 November. Maximum reduction was observed in DBW 14 (14.84%) while minimum reduction was observed in HW2045 (0.69 %). However, in some of the wheat genotypes (HS240, Raj4101, K0307 and HI1544 they show increment in grain number/spike under elevated temperatures conditions but the difference was also found non-significant (**Dhyani, 2010).** During an investigation, two varieties of wheat viz. WH730 (high temperature tolerant) and UP2565 (high temperature sensitive) were used to study the effects of high temperature (by shifting the pots to polyhouse for one week with maximum temperature 5°C–8°C > than ambient and controlled condition; field capacity (10–12 %) and ambient temperature (10°C–26°C) on growth of the crop. The results showed a 14 % reduction in mean grain number and 10 % reduction in mean grain weight under heat stress condition as compared to controlled condition. Due to heat stress, a 12 % reduction in mean grain yield was also observed. High temperature when applied at post- anthesis

stage of wheat causes shortened duration of maturation, grain filling duration and reduced grain yield, mean grain weight and grain number (**Kaur and Behel, 2010).** Every single degree elevation in temperature lead to a decrease of 4% of grain number per spike. Inadequate supply of a assimilates could also be a reason for floret sterility that led to decrement in grain number per spike. If wheat plant experience high temperature >30°C during pollen mother cells are dividing substantially, reduce grain set and therefore grain yield (**Farooq *et al*., 2011**). Heat stress also impairs viability of anther and pollen, resulting in poor fertilization. The sensitivity of pollen towards high temperature is due to they are found incapable to produce HSPs in response to heat stress. Elevated temperature during meiosis and growth of ovaries could also be as reason for reduction in grain weight in wheat crop under late sowing (**Farooq *et al*., 2011**). Heat stress also found responsible for disturbance in kernel morphology by compacting the duration of grain filling period. According to previous report on the post-anthesis heat stress on head trait of wheat showed that various parameters such as grain filling duration, head weight, kernel weight were reduced in comparison to control but kernel number remains unaffected (**Iqbal *et al.*, 2017**). An experiment was performed to analyse the effect of terminal heat stress on various yield attributes of different wheat varieties under two crop seasons planting dates, normal (mid-November) and elevated temperatures (late December) with proper irrigated environments. The results showed that due to heat stress, grain yield was the most affected trait among others (>30 % reduction), whereas grain number/spike were less affected (<15 %). Based on the performance of genotypes under both sowing conditions WH 1021, NW 1014 and NW 2036 were found as the heat tolerant wheat genotypes, while HD3086, HD 2967 and HD 3059 were suitable for both normal and heat stress environments. The reason for the difference in performance of various genotypes under both sowing conditions is may be due to 8.1 % reduction in days to anthesis and 13 % in days to maturity which cause shortening of growth phase and filling duration that ultimately led to forced senescence of crop (**Kumar *et al*., 2020**). During an experiment with 58 wheat genotypes a reduction of 30.90 % in grain number/m^2, 25.30 % in grains/spike, 13.10 in spikelet per spike, was observed under late planting on March 1, 2016, as compared to optimal planting condition on October 1, 2015. Spikelet number/spike was found more significant under heat stress than non-stressed condition.

Grain number/spike had strong positive relationship with the grain yield under non-stressed condition while under stressed condition, grain number (per spike), had strong correlation with grain yield. **E**arly sown wheat crop completes its grain filling duration before onset of high temperature thus can reduce the spikelet sterility (**Youldash *et al*., 2020).** In another experiment also spikelets per spike and number of grains per spike were found significantly reduced under heat stress (inside the plastic tunnel) condition in comparison to the wheat seeds variety Faisalabad-2008, under no stress/controlled condition (well-watered and normal condition). According to the author's results spikelets per spike under non stress condition was 18.40 while under stressful condition it reduces to 14.85 similarly grains per spike under controlled condition was found 45.32 and under heat stress condition it become 32.64. The phenomenon of reduction in spikelets per spike and grains number per spike was due to disturbance in various reproductive processes under elevated temperatures. Sometimes heat stress reduced the pollen viability, ovule fertility, spike initiation and spikelets differentiation. Along with that sometimes heat stress can enhance floret and spike concentrations of abscisic acid that might be associated with poor grain set (**Sattar *et al*., 2020**). In a recent investigation, two spring wheat cultivars (Millet-11, Punjab-11) & (V-07096, V-10110) were investigated in response to terminal heat stress. Based on their morpho-physiological traits, the results concludes that elevated temperatures cause 10 % reduction in number of grains per spike as compared to non-heat stress condition in all wheat cultivars. The reduced number of grains per spike in the present study might be due to low grain fertility and floral spikelets associated with increased temperature even by 1^o C during booting and anthesis stages. As reduction in number of grains depends on developmental stage, at which high temperature occurs and determined by supply of carbohydrates during floral development, which is a sensitive process to high temperature **(Tariq *et al*., 2021).**

4.3.4 Thousand grains weight

Thousand (1000) grains weight of different wheat varieties were recorded under both sowing condition in two consecutive years 2018-19 and 2019-20 at maturity. The results showed reduction in 1000 grains weight of different wheat varieties under D2 in both years as compared to D1. In detailed view, during 1st trial

(2018-19), the maximum 1000 grain weight was observed in UP2565 (53.07 grams) and minimum in UP2628 (46.08 grams) under D1 while under D2, 1000 grain weight was found to be reduced and maximum value was observed in UP2565 variety (42.96 grams) and minimum in HD3086 (38.16 grams). Similarly, during 2nd trial (2019-20), maximum 1000 grains weight was observed in UP2526 variety (56.50 grams) and minimum in UP2628 (46.17 grams) under D1 while under D2, maximum 1000 grain weight observed in UP2565 (45.96 grams) and minimum in UP2784 (31.73 grams). On comparing the mean values of two-year trial data (2018-19 and 2019-20), maximum 1000 grains weight was gained by UP2526 variety (54.71 grams) and minimum by UP2628 (46.12 grams) under D1. However, under D2, maximum 1000 grains weight was gained by UP2565 variety (44.46 grams) and minimum byUP2784 (33.42 grams) (**Table 4.3.4).** Over all maximum reduction in 1000 grains weight due to D2 (elevated temperatures) was recorded in UP2784 (28.21 %) while minimum in UP2748 (10.34 %) (**Figure 4.3.4**). Statistically, there was a significant difference in 1000 grain weight at the time of maturity between the two-sowing conditions (D1 and D2) and between varieties ($p \leq 0.01$) while there was a non-significant difference was found between the years ($p > 0.05$). All the interactions were also found non-significant with each other ($p > 0.05$) except the interaction between treatment × variety that was found significant (($p \leq 0.05$)

Table 4.3.4 Effect of differential temperatures on 1000 grains weight of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

1000 grain weight (grams)						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	46.08	46.17	**46.12**	39.02	37.03	**38.03**
HD3086	48.54	48.10	**48.32**	38.16	38.87	**38.51**
UP2967	49.27	50.10	**49.68**	38.68	38.23	**38.46**
UP2784	45.47	47.63	**46.55**	35.10	31.73	**33.42**
UP2526	52.92	56.50	**54.71**	41.16	43.60	**42.38**
UP2565	53.07	54.57	**53.82**	42.96	45.96	**44.46**
UP2748	47.20	46.51	**46.86**	40.36	43.67	**42.01**
HD3059	47.40	49.83	**48.62**	41.76	40.03	**40.89**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.0311		0.623		0.882	
CD (5%)	0.881		1.763		2.493	

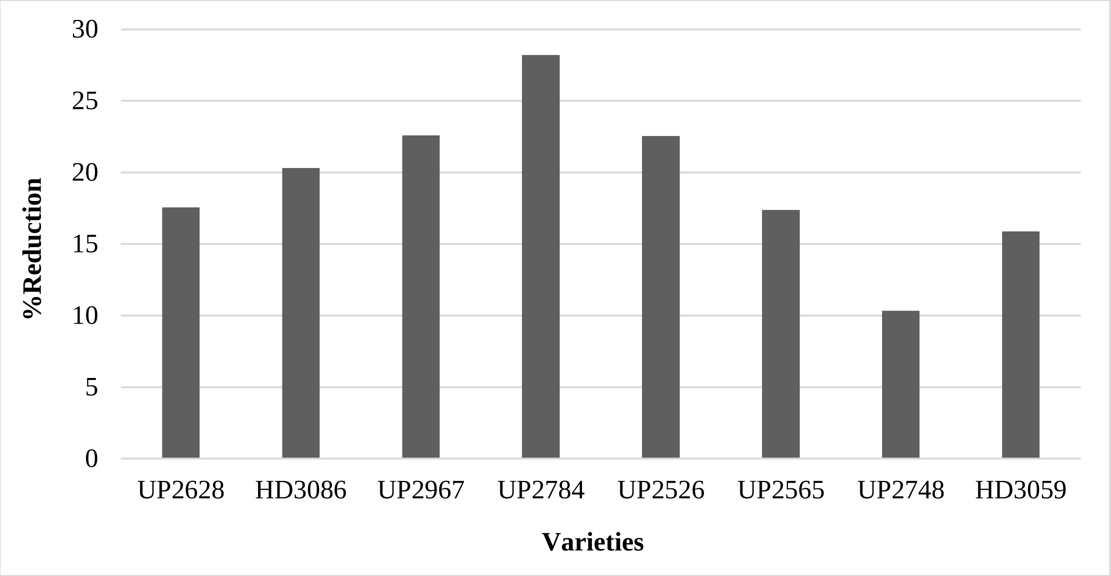


Figure 4.3.4 Reduction in mean values of 1000 grains weight of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present investigation, 1000 grain weight was found to be reduced under D2 in comparison to D1 sown condition in both years for all wheat varieties. Similar trend was also followed by previous researches in which 1000 grain weight was found reduced due to elevation in temperature. An experiment was conducted with 14 spring wheat genotypes, exposed to heat stress from 30 to 38ºC, causes reduction of grain weight by 20 to 44% (**Tahir and Nakata, 2005**). Similarly, in an experiment, 1000 grain weight was found to reduce ranging from 5.73% to 19.23% in different wheat varieties namely HD2687, UP2113, VL616, PBW343, UP2338, PBW175, UP2425 and VL421 under elevated temperatures compared to normal condition. Least reduction in 1000 grain weight was observed in UP2113 (5.73 %) **(Srivastava, 2005).** In an experiment, 1000 grain weight was found significantly reduced in different wheat genotypes sown on 23 December in comparison to wheat genotypes sown on 20 November. Maximum reduction was observed in 36PBW-373 (25.73 %) while minimum reduction was observed in 32Raj4083(I) (4.26 %). The reason for reduction in 1000 grain weight according to the author was improper grain filling as post anthesis heat stress led to affect filling of the grains very badly (**Dhyani, 2010).** Similar trend also followed by other researches in which 1000 grain weight was found reduced under elevated temperatures (19th December and 17th December) in comparison to early sowing wheat cultivar (25th November and 23rd November). A reduction from 3.99 % to 16.20 % in 1000 grain weight was observed in all wheat varieties (**Chandra, 2006).** According to the previous finding, higher temperature causes shrinking of the grains when day and night temperature exceeds from 25/14ºC to 31/20ºC leads to improper filling of the grains hence reduced 1000 grain weight. As under stress condition the packing of the endosperm cellular structure is also found very dense due to the presence of starch granules embedded in protein matrix but under non-stress condition this packing is not at all dense however, wheat grain has larger cells surrounded by starchy endosperm under non stress condition (normal sowing) **(Dias *et al*., 2008**). According to another author elevation of temperature during meiosis and growth of ovaries in wheat life cycle could also be as reason for reduction in grain weight under delayed sowing condition (**Farooq *et al*., 2011**). Heat stress also found responsible for disturbance in kernel morphology by compacting the duration of grain filling period. According to previous report on the post-anthesis heat stress on head trait of wheat showed that various parameters such as grain filling duration, head weight, kernel weight were reduced in comparison to control (**Iqbal *et al.,* 2017**).An experiment was done in which winter wheat cultivar Gaocheng 8901 was grown under natural condition (25ºC for 12 hour at day/ 18 ºC for 12 hour at night) and heat stress condition (artificial chamber with 40ºC for 2 hour treatment at the time of flowering to maturity). The results showed simultaneous decrease (42.85 %) in 1000 grains weight under stress condition as compared to normal condition (**Zang *et al*., 2018**). A study was done in 58 different wheat genotypes in which a reduction of 4.30% in grain weight was observed under late planting on March 1, 2016 as compared to optimal planting condition on October 1, 2015. Grain weight, had strong positive relationship with the grain yield under non-stressed condition while under stressed condition (**Youldash *et al*., 2020).** In an experiment the effect of terminal heat stress on various yield attributes of different wheat varieties under two crop seasons planting dates, normal (mid-November) and late sown (late December) were analysed. The results shows that due to elevated temperatures, thousand kernel weight was affected by 11.40 %. The reason for the difference in performance of various genotypes under both sowing conditions is maybe due to 8.1

% reduction in days to anthesis and 13 % in days to maturity which cause shortening of growth phase and filling duration causes reduced grain weight under elevated temperatures sowing condition (**Kumar *et al*., 2020**). In a recent investigation, spring wheat cultivars (Millet-11, Punjab-11) & (V-07096, V-10110) were investigated in response to terminal heat stress. Based on their morpho-physiological traits, the results concludes that elevated temperatures cause reduction in 1000-grain weight by 13 % **(Tariq *et al*., 2021).**

4.3.5 Biological yield (tons/hectare) and Grain yield (tons/hectare)

Biological yield and grain yield of different wheat varieties were analysed under both sowing condition in two consecutive years 2018-19 and 2019-20 at maturity. The results showed that the biological yield and grain yield was found reduced under D2 in all wheat varieties as compared to D1. In detailed view, during 1st trial (2018-19), the maximum biological yield was observed in variety HD3086 (19.17t/ha) and minimum in UP2748 (15.80 t/ha) under D1 while under D2, biological yield was found maximum in two varieties UP2628 and UP2748 (15.59 t/ha) and minimum in HD3086 (14.51 t/ha). Similarly, during 2nd trial (2019-20), maximum biological yield was observed in HD3059 (21.11 t/ha) and minimum in UP2526 (16.81 t/ha) under D1 while under D2, maximum biological yield was observed in HD3059 (17.13 t/ha) and minimum in UP2565 (13.46 t/ha). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum biological yield was gained by variety HD3059 (20.08 t/ha) and minimum by UP2526 (16.58 t/ha) under D1. However, under D2, maximum biological yield was gained for UP2628 variety (16.28 t/ha) and minimum for UP2565(13.60) (**Table 4.3.5).** Over all maximum reduction in biological yield due to D2 (elevated temperatures) was recorded in HD3086 (23.11 %) while minimum in UP2526 (6.89 %) (**Figure 4.3.5**). Statistically, a significant difference in the values of biological yield between the two-sowing conditions (D1 and D2) ($p \leq 0.01$), between two years ($p \leq 0.01$) and between varieties ($p \leq 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$).

During 1st trial (2018-19), the maximum grain yield was observed in UP2967 (7.25 t/ha) and minimum in UP2748 (4.79 t/ha) under D1 while under D2, grain yield was found to be reduced and maximum value observed in UP2628 variety (5.88 t/ha) and minimum in UP2748 (4.52 t/ha). Similarly, during 2nd trial (2019-20), maximum grain yield was observed in UP2628 variety (7.58 t/ha) and minimum in UP2748 (4.89 t/ha) under D1 while under D2, maximum grain yield observed in UP2628 (6.91 t/ha) and minimum in UP2748 (4.96 t/ha). On comparing the mean values of two-year trial data (2018-19 and 2019-20), maximum grain yield was gained by UP2628 variety (6.94 t/ha) and minimum by UP2748 (4.84 t/ha) under D1. However, under D2, maximum grain yield was gained for UP2628 variety (6.40 t/ha) and minimum for UP2748 (4.74 t/ha) (**Table 4.3.5).** Over all maximum reduction in grain yield due to D2 (elevated temperatures) was recorded in UP2784 (20.05 %) while minimum reduction in grain yield was observed in UP2526 & UP2748 (1.45 % & 2.93 %) respectively (**Figure 4.3.5**). Statistically, a significant difference in the values of grain yield between the two-sowing conditions (D1 and D2) ($p \leq 0.01$), between varieties ($p \leq 0.01$) and between two years ($p \leq 0.05$). All the other interactions were also found significant with each other ($p \leq 0.01$). However, the interaction between year × treatment × variety were found significant at ($p \leq 0.05$).

Table 4.3.5 Effect of differential temperatures on biological yield (t/ha) and grain yield (t/ha) of different wheat varieties at maturity. *Remark; (D1& D2 indicates sowing in the month of November and December respectively).*

	Biological yield (t/ha)						**Grain yield (t/ha)**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	16.11	19.86	**17.99**	15.59	16.98	**16.28**	6.31	7.58	**6.94**	5.88	6.91	**6.40**
HD3086	19.17	20.97	**20.07**	14.51	16.36	**15.43**	6.51	6.03	**6.27**	5.24	5.28	**5.26**
UP2967	16.81	19.44	**18.13**	14.66	14.66	**14.66**	7.25	4.93	**6.09**	4.98	5.15	**5.07**
UP2784	17.36	19.72	**18.54**	15.28	14.60	**14.94**	7.61	5.98	**6.80**	5.56	5.31	**5.44**
UP2526	16.36	16.81	**16.58**	15.51	16.51	**16.01**	5.59	5.46	**5.53**	5.51	5.39	**5.45**
UP2565	16.25	17.36	**16.81**	13.73	13.46	**13.60**	6.06	5.89	**5.97**	5.85	5.65	**5.75**
UP2748	15.80	17.50	**16.65**	15.59	15.43	**15.51**	4.79	4.89	**4.84**	4.52	4.96	**4.74**
HD3059	19.06	21.11	**20.08**	14.66	17.13	**15.90**	6.74	5.43	**6.08**	5.34	5.39	**5.36**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.073		0.146		0.206		0.017		0.034		0.0489	
CD (5%)	0.206		0.413		0.584		0.048		0.097		0.138	

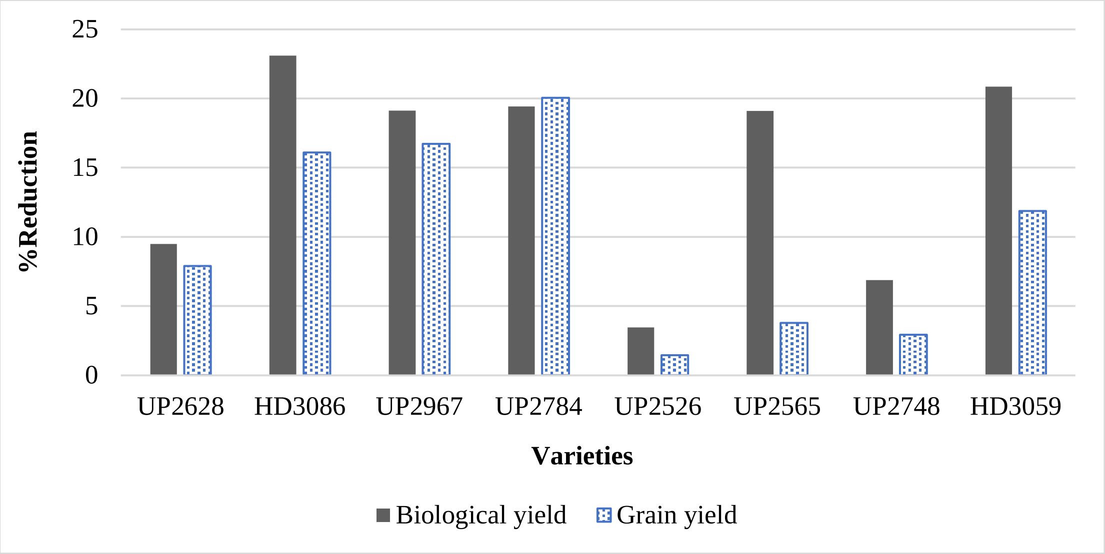


Figure 4.3.5 Reduction in mean values of biological yield and grain yield of different varieties under D2 as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present investigation, biological and grain yield was found reduced under D2 (elevated temperatures) as compared to D1 in both the years. Similar results were also followed by previous scientists in which biomass and grain yield was found negatively affected by elevated temperatures. A reduction of 32.90 % to 38.57 % in different wheat varieties namely HD2687, UP2113, VL616, PBW343, UP2338, PBW175, UP2425 and VL421 was observed under D2 as compared to D1. Least reduction in grain yield was observed in PBW175 (32.90 %) while maximum reduction in grain yield observed in VL421 (38.57 %). The reduced grain yield under elevated temperatures is due to warmer temperature, hasten the development, shorten the growth period, thus finally lowers the grain yield along with that under elevated temperature this accelerates the respiratory process in plants causes inhibition in dry matter accumulation in sink hence biological yield also reduces (**Srivastava, 2005).** In an experiment 14 spring wheat genotypes were exposed to heat stress from 30 to 38 ºC, causes reduction of grain weight by 20 to 44 % (**Tahir and Nakata, 2005**). In another experiment, grain yield was found reduced under late sown condition on 19th December and 17th December in different wheat cultivars in comparison to early sown wheat cultivar on 25th November and 23rd November. A reduction from 10.72 % to 24.71 % in grain yield was observed in all wheat varieties. The warmer temperature causes inappropriate growth, development and grain filling and results reduction in number of grains per spike (**Chandra, 2006)**. In a research, average biological yield under timely sown condition was found 9.82 tons/ha while under late sown condition the average biological yield reduced to 6.65 tons/ha. Similarly average grain yield under optimum sowing conditions was found 4.10 tons/ha while under elevated temperatures conditions, the average grain yield reduced to 2.62 tons/ha (**Dhyani, 2010).** In an experiment the optimum date of sowing for wheat variety UP-2565 was analysed in three different dates (1 Dec, 20 Dec and 6th Jan of year 2014-15 and 2015-16). The results showed that the crop sown under late sown conditions had less grain yield and biological yield as compared to timely sown condition along with that under late sowing, the crop took less number of days to attain maturity. Maximum reduction in both yield data was found for the crop sown on 6th January as compared to other dates of sowing. This reduction in the biological yield is due to higher temperature results in reduction in duration of completion of different physiological parameters and thus crop get less time for dry matter accumulation. Delay in sowing also causes reduction of grain yield probably because of exposure of crop to high temperature which reduces length of growing duration. On comparing the two years data reduction in dry matter, grain yield and biological yield was more in the year 2015-16 in comparison to 2014-15 probably because of high temperature at the reproductive stage during the crop growing season (**Gupta *et al*., 2017**). Through an experiment, the effect of terminal heat stress on various yield attributes of different wheat varieties under two crop seasons planting dates, normal (mid-November) and late sown (late December). The results shows that due to heat stress, grain yield was the most affected trait among others (>30 % reduction). The difference in performance of various genotypes under both sowing conditions may be due to shortening of growth phase and filling duration that ultimately led to forced senescence of crop (**Kumar *et al*., 2020**). A study was performed in 58 different wheat genotypes in which a reduction of 8.4% in biological yield and 33.90 % in grain yield was observed when wheat genotypes planted on March 1, 2016as compared to optimal planting condition (October 1, 2015) (**Youldash *et al*., 2020).** In a recent investigation, spring wheat cultivars (Millet-11, Punjab-11) & (V-07096, V-10110) were investigated in response to terminal heat stress (due to Elevated temperatures condition). Based on their morpho-physiological traits, the results concludes that Elevated temperatures causes reduction in biological yield (15–20 %) in comparison to normal conditions in all wheat cultivars. However, higher grain yield (9 %) was recorded under elevated temperatures. According to the research, Punjab-11 found as better agronomic performance as compared to other cultivars as it has delayed maturity with proper grain filling period **(Tariq *et al*., 2021).**

4.3.6 Harvest Index (%)

Harvest index of different wheat varieties was calculated under both sowing condition in two consecutive years 2018-19 and 2019-20 at maturity. The results showed no general trend in the values of harvest index under D2in comparison to D1. For wheat varieties UP2628 and UP2784, harvest index was found to be reduced due to D2 (elevated temperatures) while in rest of varieties it is increasing under D2. In detailed view, during 1st trial (2018-19), the maximum HI recorded in UP2784 (45.92 %) and minimum in UP2748 (31.04 %) underD1 while under D2, maximum HI recorded in UP2565 variety (42.61 %) and minimum in UP2748 (31.85 %). Similarly, during 2nd trial (2019-20), maximum HI recorded for UP2628 (39.24 %) and minimum for UP2967 (25.39 %) under D1 while under D2 maximum HI recorded in UP2565 (42.01 %) and minimum in UP2784 (31.29 %). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum HI was achieved by UP2628 variety (39.20 %) and minimum by UP2748 (29.47 %) under D1. However, under D2 maximum HI experienced by UP2565 variety (42.31%) and minimum for by UP2748 (32.08 %) (**Table 4.3.6).** Over all the reduction in HI due to D2 (elevated temperatures) was recorded in UP2628 and UP2784 variety (1.38 % and 12.40 % respectively) while a maximum increment in HI was observed in UP2967 (17.97 %) and minimum increment was observed in UP2526 (2.08 %) (**Figure 4.3.6**). Statistically, a significant difference in the HI between the between two years and between the varieties at ($p \leq 0.01$) level of significance while between the two sowing conditions (D1 and D2) &was found non-significant ($p > 0.05$). All the other interactions were also found non-significant with each other ($p > 0.05$).

Table 4.3.6 Effect of differential temperatures on harvest index (%) of different wheat varieties at maturity. *(D1& D2 indicates sowing in the month of November and December respectively).*

Harvest Index (%)						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	39.16	39.24	**39.20**	38.76	38.57	**38.66**
HD3086	33.99	28.75	**31.37**	36.33	32.26	**34.30**
UP2967	34.14	25.39	**29.77**	34.84	35.40	**35.12**
UP2784	45.92	31.53	**38.72**	36.52	31.29	**33.91**
UP2526	34.18	32.98	**33.58**	35.56	33.00	**34.28**
UP2565	37.61	35.25	**36.43**	42.61	42.01	**42.31**
UP2748	31.04	27.90	**29.47**	31.85	32.31	**32.08**
HD3059	35.45	25.96	**30.70**	36.41	31.47	**33.94**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	1.546		1.093		2.186	
CD (5%)	2.185		4.371		6.182	

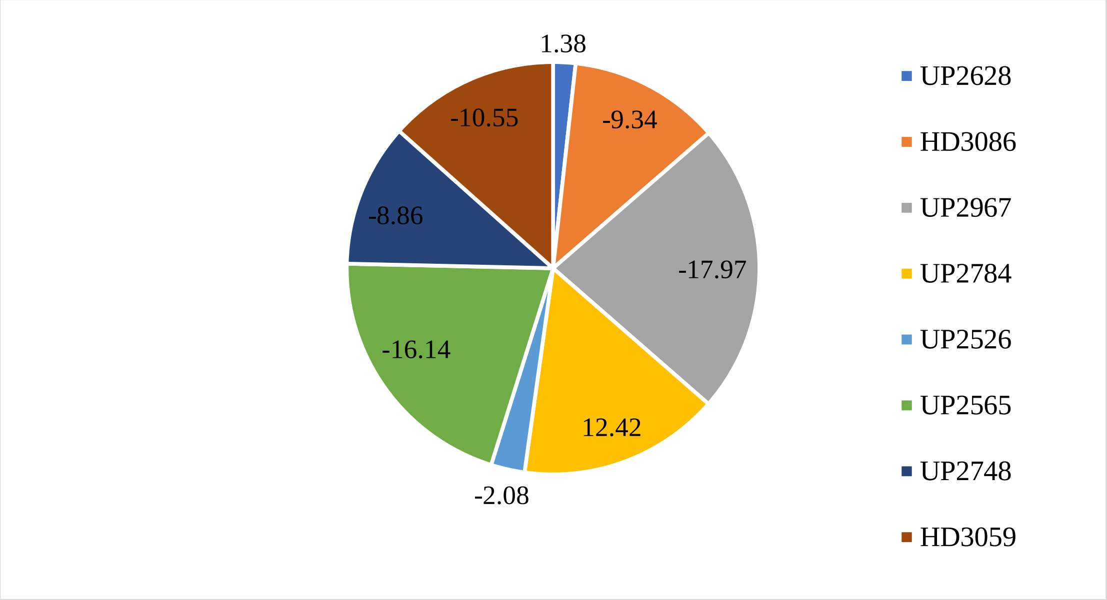


Figure 4.3.6 Reduction in mean values of harvest index of different varieties under D2 as compared to D1. (*Note*- "Negative" sign represent percent increase in values of harvest index), (D1& D2 indicates sowing in the month of November and December respectively).*

In present research no general trend was observed for harvest index in different wheat varieties. HI was found reduced for UP2628 and UP2784 wheat varieties under D2 in comparison to D1 while for wheat variety, HD3086, UP2967, UP2526, UP2565, UP2748 and HD3059, harvest index was found to be increased. Reduction in HI under elevated temperature was recorded in past researches in which HI was found to reduce from 31.80 to 42.32 % in different wheat varieties namely HD2687, UP2113, VL616, PBW343, UP2338, PBW175, UP2425 and VL421 as compared to optimum conditions. The heat stress causes reduction in vegetative and reproductive growth phases in plants hence reduces grain yield and biological yield of crop and eventually decline the portioning of biomass from vegetative to reproductive organ which reduced harvest index i.e grain spike ratio **(Srivastava, 2005).** Similar results were also observed in another experiment in which harvest index was found reduced under elevated temperatures when wheat cultivars sown on 19th December and 17th December in comparison to cultivars sown on 25th November and 23rd November, a reduction from 6.22 % to 31.34 % under elevated temperatures was observed (**Chandra, 2006).** Harvest index was found more under normal sown condition (20 November) (41.79 %) than elevated temperature conditions (December 23) (38.83 %) (**Dhyani, 2010**). However, harvest index also used to estimate crop carbon balancing by applying it to grain yield statistics to determine total shoot dry matter and then calculating crop residues as the difference between shoot carbon and grain carbon. As heat stress, shortens the length of the vegetative phase can sometimes shows increment in HI. For wheat, or some other C3 cereals, due to the presence of some other nutrient in excess such as nitrogen, can enhance the allocation of photosynthate to structural carbon, which cannot be mobilized to grain later, resulting in a decrease in HI (**Unkovich *et al*., 2010**). An experiment was performed with 80 wheat genotypes (20 each from *Triticum aestivum, Triticum durum, Triticum dicoccum and Synthetic T. aestivum*) for analysing their performances for terminal heat tolerance by normal (non-stress) and late (elevated temperatures) planting conditions. The results showed that genotypes of wheat from *Triticum aestivum, Triticum durum and Synthetic T. aestivum* showed reduced harvest index % under heat stress condition as compared to normal conditions but for *Triticum dicoccum* genotypes, showed increment in harvest index under heat stress conditions in comparison to normal planted. During optimum sown plantation, HI was (16.80 ± 1.81%) but under elevated temperature plantation it was increased to 17.82±1.28 % (**Kavita *et al*., 2016**). A study was done to reveal the effect of elevated temperatures on yields and yield attributed of new soybean cultivars developed for warm regions. The experiment was conducted in two consecutive years 2016 and 2017 in Japan. They were planted in June (normal) and July (heat stress). The results showed that under heat stress conditions, harvest index was significantly increases from 0.464 to 0.571 in 2016 and from 0.524 to 0.585 in2017. In this study HI was found correlated with stem dry weight rather than seed yield. Thus, it could be the possible reason for stabilize yield of soyabean in southwestern Japan with higher HI under elevated temperatures **(Kawasaki *et al* 2018**). Another experiment was done with six wheat (Triticum aestivum L.) cultivars (C306, HD2285, HD2329, Kundan, HD2643 and PBW343) which was sown on three different dates (15th November, 15th December and 15th January) to examine the effect of terminal heat stress on growth, yield and yield attributing characters. The results showed that HI was found decreased for some cultivars as date of sowing increases while in some cultivars with increasing date of sowing, HI% decreases. For example, in cultivar HD2329, Kundan, HD2643 and PBW343 HI% found higher HI% under 15th December (late sowing) as compared to 15th November (normal sowing). In cultivar C306, HI % also found higher HI % under 15th January (very late sowing) as compared to 15th December (late sowing). Rest of the cultivars showed general trend of reduced HI% under late and very late sowing condition in comparison to normal. This difference in results was may be due to individual cultivar performance with respect to differential response of grain to shoot weight ratio with increases sowing date (**Singh and Yadav, 2019**). In a recent study which was done with 58 different wheat genotypes a reduction of 28% in harvest index in different wheat varieties was observed under planting date (March 1, 2016) as compared to optimal planting condition (October 1, 2015) (**Youldash *et al*., 2020).** Harvest index (HI) is a measure of reproductive efficiency which depends on the ratio of grain to total shoot dry matter and it is determined by interactions between genotypes (G), environment (E), and crop management (M) so the performance of HI under late plantation may be depend on various other factors in which environmental factors is most important which includes seasonal pattern of water supply and extreme temperatures during crop reproductive development **(Poker, 2020).**

4.3.7 Heat susceptibility Index for grain yield.

Heat susceptibility Index for grain yield was calculated for each variety and the results showed differential response in the values of H.S.I for different varieties. The wheat varieties UP2628, UP2526, UP2565 and UP2748 possess lower heat susceptibility index (showed tolerance towards heat stress) as compared to HD3086, UP2967, UP2784 and HD3059 which was found susceptible for elevated temperatures which is according to the data provided by calculating heat susceptibility index for grain yield. Lowest H.S.I was observed in UP2748 and highest H.S.I was recorded in UP2784 variety (**Table 4.3.7).**

Table 4.3.7 Effect of differential temperatures on heat susceptibility Index for grain yield of different wheat varieties. *(D1& D2 indicates sowing in the month of November and December respectively).*

Varieties	Heat Susceptibility Index (H.S.I)
UP2628	0.90
HD3086	1.29
UP2967	1.32
UP2784	1.48
UP2526	0.60
UP2565	0.70
UP2748	0.38
HD3059	1.09

Heat susceptibility Index (HSI) is used to determining the stress tolerance level of particular crop under unfavourable and favourable conditions (**Asana and Williams, 1965).** During present investigation value of H.S.I was found different for different varieties. Previous researches showed that the genotypes having high positive value of HSI are susceptible to higher temperatures and those having low H.S.I are tolerant towards heat stress. Grain weight reduction is one of the best measures for idolizing the heat tolerance behaviour in wheat, so with the help of heat susceptibility index for grain yield per genotype/ variety is an effective measure to evaluate the thermo-tolerance characteristic of various genotypes (**Mahammadi 2004**). An experiment was conducted with 80 wheat genotypes (20 each from *Triticum aestivum, Triticum durum,Triticumdicoccum* and *Synthetic T. aestivum*) for analysing their performances for terminal heat tolerance by normal (non-stress) and late (elevated temperatures) planting conditions. The results showed different values of heat susceptibility index for different wheat genotypes. In brief, genotypes PBW 550, HD 2733, HD 2967, PBW 343 & PBW373 (T. *aestivum*), WHD 943, WHD 945, WHD 314, WHD 946 & WHD912 (T. durum), DI 26, DI 119, DI 88, DI 52 & DI 61 (T. *dicoccum)* and Syn 22, Syn 24, Syn 30, Syn 36, Syn 62 & Syn 25 (Synthetic T. *aestivum*) were found heat tolerant on the basis of low heat susceptibility index and could be useful as genetic stock to develop wheat heat tolerant varieties in breeding programs. Minimum HSI was found in the genotypes PBW 550 (0.19) in T. *aestivum*; WHD 943 (0.14) in T. durum; DI 26 (0.28) in T. *dicoccum* and SYN 67 and SYN 36 (0.44) in Synthetic T. *aestivum* (**Kavita *et al*., 2016**). Another experiment was done in wheat under normal & heat stress conditions were sown at field level, in which genotypes Raj 3765, WH 1080 and WH 1142 showed minimum HSI for a number of traits and almost all genotype of triticale group have minimum values of HSI representing high temperature tolerance of these genotypes (**Suresh *et al*., 2018**). In a recent study it was concluded that the wheat genotypes; Altınözü (1.28), Axe (1.29), Eagle Rock (1.35), Osmaniyem (1.24) and Sagittatio (1.32) were found sensitive to higher temperatures with higher values of H.S.I. while, genotypes Alibey (0.68), Genç99 (0.56) and Kayra (0.70) were found resistant towards heat stress (having lower H.S.I). Similarly, genotypes Shirogane Kamugi (0.05) and Chukogu-146 (0.058) were more resistant (having lowest value of H.S.I) to heat stress due to early maturity (**Youldash *et al*., 2020)**.

Correlation analysis between yield and yield related attributes under both sowing condition

Correlation was analysed for yield and yield attributes under both sowing conditions. Under D1, grain yield was positively correlated with biological yield, harvest index, total spike number and total spike weight and negatively correlated with thousand grain weight. While under D2, grain yield was positively correlated with biological yield, harvest index, total spike weight and negatively correlated with thousand grain weight as well as with total spike number. The correlations under each sowing condition were non-significant. Negative correlation of grain yield with two parameters (thousand grain weight and total spike number) under elevated temperatures could be one of possible reasons of reduced yield and weight under D2in comparison to D1 where negative correlation of grain yield was found with only one parameter (thousand grain weight).On comparing the other parameters of yield attributes, positive and significant correlation was observed between total spike number and total spike weigh (r=0.77*) while negative but non-significant correlation was observed between biological yield & all parameters and harvest index with thousand grain weight under D1. However, under D2, biological yield also had negative but non-significant correlation with other parameters except with total spike weight which had non-significantly positive correlation. Harvest index had positive correlation with thousand grain weight and total spike weight while negatively correlated with total spike number. Thousand grain weight negatively correlated with total spike number and weight. From this data it can be concluded that under both sowing conditions some yield parameters were found negatively correlated with each other but more negative correlations between yield parameters were observed under D2 as compared to D1(**Table 4.3.8.1 and Table 4.3.8.2).**

Table 4.3.8.1 Pearson's correlation between yield and yield related attributes under D1.

	GY	*BY*	*HI*	*TGW*	*TSN*	*TSW*
GY	1					
BY	0.55	1				
HI	0.69	-0.11	1			
TGW	-0.35	-0.45	-0.07	1		
TSN	0.14	-0.32	0.39	0.13	1	
TSW	0.11	-0.38	0.24	0.55	0.77*	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Biological yield (BY), Harvest Index (HI), Thousand Grain Weight (TWG), Total Spikes Number (TSN) and Total Spikes Weight (TSW).

Table 4.3.8.2 Pearson's correlation between yield and yield related attributes under D2.

	GY	*BY*	*HI*	*TGW*	*TSN*	*TSW*
GY	1					
BY	0.17	1				
HI	0.69	-0.50	1			
TGW	-0.05	-0.11	0.33	1		
TSN	-0.08	0.12	-0.39	-0.59	1	
TSW	0.15	-0.43	0.30	-0.29	0.28	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Biological yield (BY), Harvest Index (HI), Thousand Grain Weight (TWG), Total Spikes Number (TSN) and Total Spikes Weight (TSW).

4.4 Developmental Studies

4.4.1 Days to heading and Days to anthesis

The number of days taken to heading and days taken to anthesis from date of sowing was recorded for each variety under both sowing condition in two consecutive years 2018-19 and 2019-20. The results showed that under D2, number of days taken to heading and number of days taken to anthesis was found reduced as compared to the days taken to heading and anthesis under D1 by all wheat varieties. In detailed view, during 1st trial (2018-19), the maximum number of days to heading was recorded in HD3059 variety (89.33 days) and minimum in UP2748 and UP2526 (83.67 days) under D1 while under D2, number of days to heading was found reduced for all the wheat varieties and maximum recorded in UP2628 variety (82.33 days) and minimum in HD3086 (77.33 days). Similarly, during 2nd trial (2019-20), the maximum number of days to heading was recorded for UP2967 (89.00 days) and minimum for HD3086 and UP2526 (82.00 days) under D1 while under D2, number of days to heading reduced and maximum days recorded in UP2967 (81.00 days) and minimum in UP2748 (75 days). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum number of days to heading was taken by HD3059 variety (88.83 days) and minimum by UP2526 (82.83 days) under D1. However, under D2, maximum number of days for heading was taken by

UP2628 variety (81.50 days) and minimum by HD3086 and UP2784 (76.50 days) (**Table 4.4.1).** Over all the maximum reduction in number of days to heading due to D2 (elevated temperatures) was recorded in HD3059 (10.32 %) while a minimum reduction was observed in UP2628 (4.86 %) (**Figure 4.4.1**). Statistically, there was a significant difference in the number of days to heading between the two years, between the two sowing conditions and between the varieties at ($p \leq 0.01$) level of significance. All the other interactions were also significant with each other at ($p \leq 0.01$) except the interactions between year× treatment and year × treatment × varieties were found non-significant with each other at ($p > 0.05$).

For number of days taken to anthesis from date to sowing, during 1st trial (2018-19), the maximum number of days taken to anthesis was recorded in HD3059 variety (99.67 days) and minimum in HD3086 (91.33 days) under D1 while under D2, number of days taken to anthesis was found reduced for all varieties and maximum recorded in UP2565 variety (92.67 days) and minimum in HD3086 (83.00 days). Similarly, during 2nd trial (2019-20), the maximum number of days taken to anthesis was recorded for UP2967 and HD3059 (98.67 days) and minimum for HD3086 (88.33 days) under D1 while under D2, number of days taken to anthesis reduced and maximum days recorded in UP2565 (92.33 days) and minimum in UP2748 (80.67 days). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum number of days to anthesis take by HD3059 variety (99.17 days) and minimum by HD3086 (89.83 days) under D1. However, under D2, maximum number of days for anthesis was taken by UP2565 variety (92.50 days) and minimum for by HD3086 (82.00 days) (**Table 4.4.1).** Over all the maximum reduction in number of days to anthesis due to D2 (elevated temperatures) was recorded in HD3059 (13.61 %) while a minimum reduction in number of days taken to anthesis was observed inUP2565 (3.48 %) (**Figure 4.4.1**). Statistically, there was a significant difference in the number of days to anthesis between the two years, between the two sowing conditions and between the varieties at ($p \leq 0.01$) level of significance. All the other interactions were also significant with each other at ($p \leq 0.01$) except the interactions between year× treatment and year × treatment × varieties were found non-significant with each other at ($p > 0.05$).

Table 4.4.1 **Effect of differential temperatures on number of days taken to heading and anthesis from date of sowing of different wheat varieties.** *(D1& D2 indicates sowing in the month of November and December respectively).*

	Days to Heading						**Days to Anthesis**					
Varieties	**D1**			**D2**			**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	87.33	84.00	**85.67**	82.33	80.67	**81.50**	94.00	90.67	**92.33**	88.33	86.67	**87.50**
HD3086	84.67	82.00	**83.33**	77.33	75.67	**76.50**	91.33	88.33	**89.83**	83.00	81.00	**82.00**
UP2967	88.33	89.00	**88.67**	81.67	81.00	**81.33**	98.00	98.67	**98.33**	88.67	87.67	**88.17**
UP2784	86.67	86.67	**86.67**	78.67	78.67	**78.67**	93.33	93.00	**93.17**	84.67	84.33	**84.50**
UP2526	83.67	82.00	**82.83**	78.00	76.67	**77.33**	94.33	93.33	**93.83**	87.33	84.33	**85.83**
UP2565	88.00	88.33	**88.17**	80.67	80.33	**80.50**	95.00	96.67	**95.83**	92.67	92.33	**92.50**
UP2748	83.67	83.00	**83.33**	78.00	75.00	**76.50**	92.00	92.33	**92.17**	83.67	80.67	**82.17**
HD3059	89.33	88.33	**88.83**	80.33	79.00	**79.67**	99.67	98.67	**99.17**	86.00	85.33	**85.67**
	Treatment (T)		**Variety (V)**		**(TXV)**		**Treatment (T)**		**Variety (V)**		**(TXV)**	
SEm ±	0.139		0.278		0.393		0.162		0.325		0.460	
CD (5%)	0.392		0.785		1.11		0.459		0.919		1.300	

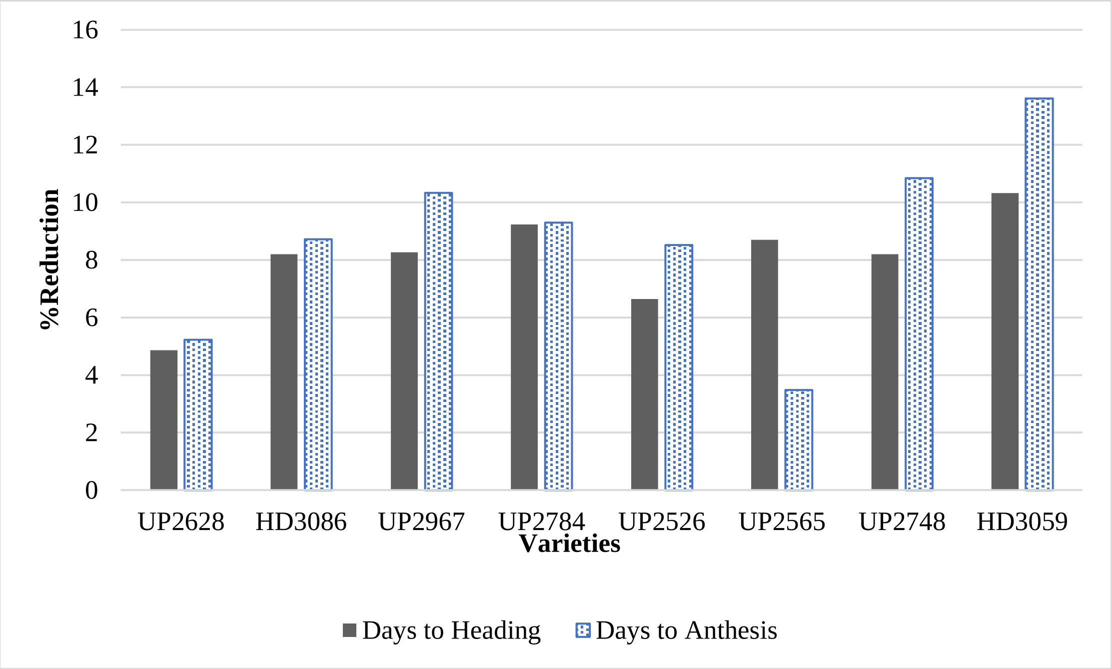


Figure 4.4.1 **Reduction in mean values of days to heading and days to anthesis from the date of sowing of different varieties under D2 as compared to D1.** *(D1& D2 indicates sowing in the month of November and December respectively).*

4.4.2 Days to Physiological Maturity/ Total life span

The number of days taken to complete the life cycle/physiological maturity /the total life span of different wheat varieties from date of sowing was calculated under both sowing condition in two consecutive years 2018-19 and 2019-20. The results showed that under D2number of days taken to physiological maturity by different varieties was found reduced as compared to D1. In detailed view, during 1st trial (2018-19), the maximum life span was recorded in UP2628, UP2784 and UP2748 variety (142.00 days) and minimum in HD3059 (135.33 days) under D1 while under D2, number of days to physiological maturity was found reduced for all varieties and maximum recorded in UP2565 variety (122.00 days) and minimum in UP2784 (114.33 days). Similarly, during 2nd trial (2019-20), the maximum number of days to physiological was recorded for UP2565 (145.33 days) and minimum for HD3059 (136.67 days) under D1 while under D2, number of days to physiological maturity reduced and maximum days recorded in UP2565 (121.00 days) and minimum in UP2628 (114 days). On comparing the mean values of two-year trial data (2018-19 and 2019-20), it was observed that maximum number of days to physiological maturity take by UP2565 and UP2748 variety (143.33 days) and minimum by HD3059 (136.00 days) under D1. However, under D2maximum number of days for physiological maturity was taken by UP2565 variety (121.50 days) and minimum for by UP2784 and UP2748(114.67 days) (**Table 4.4.2).** Over all the maximum reduction in number of days to physiological maturity due to D2 (elevated temperatures) was recorded in UP2748 (20.00%) while a minimum reduction in number of days taken to physiological maturity was observed in HD3059 (12.25%) (**Figure 4.4.2**). Statistically, there was a significant difference in the number of days to physiological maturity between the between the two sowing conditions and between the varieties at ($p \leq 0.01$) level of significance while non-significant difference was found between the two years ($p > 0.05$). All the other interactions were also found non-significant with each other at ($p > 0.05$) except the interactions between treatment × varieties that were found significant with each other at ($p \leq 0.01$).

Table 4.4.2 Effect of differential temperatures on number of days taken to physiological maturity from date of sowing of different wheat varieties. *(D1& D2 indicates sowing in the month of November and December respectively).*

Days to Physiological Maturity						
Varieties	**D1**			**D2**		
	2018-19	**2019-20**	**Mean**	**2018-19**	**2019-20**	**Mean**
UP2628	142.00	140.33	**141.17**	116.33	114.00	**115.17**
HD3086	140.00	139.00	**139.50**	117.00	118.67	**117.83**
UP2967	139.00	141.33	**140.17**	116.33	118.33	**117.33**
UP2784	142.00	143.33	**142.67**	114.33	115.00	**114.67**
UP2526	141.33	142.33	**141.83**	118.00	117.33	**117.67**
UP2565	141.33	145.33	**143.33**	122.00	121.00	**121.50**
UP2748	142.00	144.67	**143.33**	114.67	114.67	**114.67**
HD3059	135.33	136.67	**136.00**	119.00	119.67	**119.33**
	Treatment (T)		**Variety (V)**		**(TXV)**	
SEm ±	0.277		0.554		0.784	
CD (5%)	0.783		1.567		2.217	

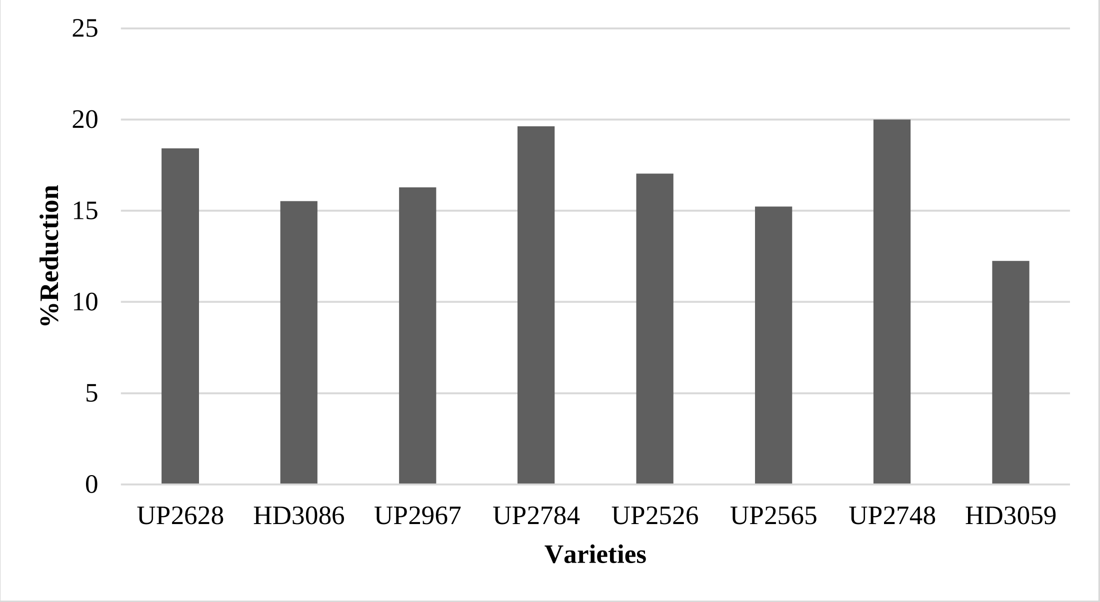


Figure 4.4.2 Reduction in mean values of days to physiological maturity from the date of sowing of different varieties under D2as compared to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present study, days to heading, days to anthesis and days taken to physiological maturity were found reduced under D2 as compared to D1 in both years. In previous researches also it was observed that in optimum sown wheat varieties, higher number of days for anthesis and maturity than when varieties sown under elevated temperatures, a delay of 30 days in sowing timing of wheat could significantly reduce the period from sowing to anthesis, anthesis to maturity and total life of wheat **(Virender & Sharma, 2000).** Delay in sowing causes exposure of plant to high temperature (heat stress) during its anthesis and grain- filling stage (which is considered as sensitive stages of wheat towards heat stress). This causes induction of forced senescence of foliage, poor assimilate synthesis, reduced translocation of photosynthates to the developing grains and great respiratory loss, ultimately affects the productivity and quality of the crop. This happens because vegetative phase of wheat life cycle is normally under control of low temperature and less humidity but as reproductive phase of wheat begins the temperature starts rising (in case of late sowing) so plant experience the exposure of high temperature (heat stress) during its anthesis and grain- filling stage **(Kanchan and Mehendra, 2000).** Normally it is believed that the greater number of days for heading and maturity favours better translocation for photosynthates from source to sink. However, when crops are under late sowing, shortened flowering period and grain filling period causes forced to flower and mature early as a result grain yield reduces **(Sikder and Hossain *et al.*, 2001).** Similar trend also followed by various research, an experiment was conducted in which different wheat varieties under two sowing conditions i.e. 27th November) and 20th December. The results showed that days to anthesis, days to maturity and total life span were reduced under 20th December as compared to 27th November sown cultivars. Under appropriate sowing condition (no heat stress), maximum number of days taken to anthesis observed in VL616 (101 days) and minimum in PBW175 (85 days). Maximum number of days taken to maturity observed in UP2113 (45 days) and minimum in PBW175 (24 days). Total life span with maximum number of days found in UP2113 (138 days) and minimum in PBW175 (109 days). However, under heat stress conditions, the number of days for each stage was reduced. Days to anthesis maximum in VL616 (91 days) and minimum in PBW175 (79 days). Days to maturity found maximum in VL421 (22 days) and minimum in PBW175 (12 days). While total life span with maximum number of days found in UP2113 (117 days) and minimum in PBW175 (91 days). The reason for such reduction according to the author is due to high temperature which causes forced senescence in cultivar and reduces overall phenological data **(Srivastava, 2005).** Similar study in whichdays taken to complete anthesis, maturity and total life span was also found reduced under 19th December and 17th December in comparison to sowing wheat cultivar on 25th November and 23rd November. Under 19th December and 17th December sown condition, maximum number of days taken to anthesis observed for PBW373 (101 days) and minimum in C306 (90 days). Maximum number of days taken to maturity observed in VL738 (32 days) and minimum in PBW373 (24 days). Total life span with maximum number of days found in VL738 (130 days) and minimum in UP1109 (113 days). However, under 19th December and 17th December sown condition, the number of days for each stage was reduced. Days to anthesis maximum in PBW373 (93 days) and minimum in UP2382 (85 days). Days to maturity found maximum in UP2382 (24 days) and minimum in UP1109 (15 days). While total life span with maximum number of days found in VL738 (113 days) and minimum in UP1109 (103 days) (**Chandra, 2006)**. In a research, 36 different wheat genotypes were analysed under two sowing condition 20 November and 23 December in order to check the effect of sowing condition on developmental study of wheat life cycle. The results showed that days taken to heading, anthesis and physiological maturity was found reduced under December sowing as compared to November sowing in all wheat varieties. Average days taken to heading under November sowing was found (79 days) as compared to December sowing conditions (74 days). Average days taken to anthesis under November sowing condition was found (90days) as compared to December sowing (84 days). Similarly, average days taken to maturity under November sowing was found 120days while under December sowing, it reduced to 109 days. According to the author when plants experiences heat stress they accelerate its growth, development and number of days taken to complete their life cycle which ultimately decreases proper development and grain filling duration and ultimately yield (**Dhyani, 2010).** According to a research, elevated temperatures during post anthesis stage that significantly reduced the duration of grain filling by 1-2 weeks showing heat reduces the duration of filling wheat grain (**Farooq *et al.*, 2011).** So, from past reports, it can be concluded that no heat stress/elevated temperature conditions had longer post heading period, longer grain filling period than heat stressed conditions along with that early sowing cultivars faces their grain filling period with low air temperature as compared to late sown wheat cultivars (**Iqbal *et al*., 2017**). In a research, delay in sowing conditions in wheat results filling duration post-ponded by 2 -3 weeks due to the increase of 2ºC in average temperature during grain filling stage. However, general filling rate i.e grain weight divided by filling days was found not affected by elevated temperatures (**Wang *et al*., 2018).** Chinese spring wheat when subjected to heat stress, showed the grains under heat stress reaches to maturity by 30 days from flowering while under control condition grain reaches to maturity by 38 days after flowering. A shortage of 8 days was observed (**Wang *et al*., 2018).** Through a recent experiment two spring wheat lines were sown under earlier plantings and late planting. The earliest planting results 124.7 days from planting to reach maturity while the delay in planting only required 104.7 days. The difference of 20 days results 14.0 more days to emerge than late plantation and 6.6 days longer to progress from emergence to maturity. The longer duration from emergence to anthesis and from anthesis to maturity for the earlier planted treatments included vegetative growth periods up to 3 days longer, and grain fill periods up 4 days longer than the late planted treatments. The reason for such difference was under early planting, plants emerged slowly, with increased total length of time to reach anthesis and maturity along with cool soil temperature during anthesis results no heat stress during that period **(Collier *et al*., 2021).**

4.5 Effect of elevated temperatures on biochemical parameters

4.5.1 Total Chlorophyll

The total chlorophyll, chlorophyll ‘a’&‘b’ and chlorophyll a/b ratio of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling).The total chlorophyll content in all the varieties was found to be reduced due to D2 (elevated temperatures) as compared to D1. On comparing between the two stages, total chlorophyll in all the varieties was found higher at the time of anthesis and tends to reduce as varieties moves towards grain filling stage. In detailed view, at anthesis highest chlorophyll was observed in UP2967 variety (2.92 mg/g) and lowest in HD3086 (2.58 mg/g) under D1 while under D2, total chlorophyll was found to be reduced and highest value observed in HD3059 (2.65 mg/g) and lowest in UP2628variety (1.89 mg/g) (**Table 4.5.1).** Maximum reduction in total chlorophyll content due to D2 (elevated temperatures) was recorded in UP2628 (31.38 %) while minimum in UP2748 (3.97 %) (**Figure 4.5.1**). Statistically values of total chlorophyll at the time of anthesis were found significantly affected by difference in sowing timings in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatment × varieties was also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest chlorophyll was observed in UP2967 variety (2.44 mg/g) and lowest in HD3086 (2.13 mg/g) under D1 while under D2, highest value of total chlorophyll was observed in UP2565 and UP2748 (2.18 and 2.17 mg/g respectively) and lowest in UP2628 variety (1.43 mg/g) (**Table 4.5.1).** Maximum reduction in total chlorophyll content at the time of grain filling due to D2 (elevated temperatures) was recorded in UP2628 (40.88 %) while minimum in UP2748 (3.98 %) (**Figure 4.5.1**). Statistically, values of total chlorophyll at the time of grain filling was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties was also found significant at ($p \leq 0.01$).

Table 4.5.1 Effect of differential temperatures on total chlorophyll (mg/g FW) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="9">Total chlorophyll (mg/g FW)</th></tr>
<tr><th colspan="5">At anthesis</th><th colspan="4">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th colspan="2">D2</th><th colspan="2">D1</th><th colspan="2">D2</th></tr>
<tr><td>UP2628</td><td colspan="2">2.76</td><td colspan="2">1.89</td><td colspan="2">2.42</td><td colspan="2">1.43</td></tr>
<tr><td>HD3086</td><td colspan="2">2.58</td><td colspan="2">2.35</td><td colspan="2">2.13</td><td colspan="2">1.71</td></tr>
<tr><td>UP2967</td><td colspan="2">2.92</td><td colspan="2">2.57</td><td colspan="2">2.44</td><td colspan="2">1.83</td></tr>
<tr><td>UP2784</td><td colspan="2">2.82</td><td colspan="2">2.48</td><td colspan="2">2.23</td><td colspan="2">2.02</td></tr>
<tr><td>UP2526</td><td colspan="2">2.78</td><td colspan="2">2.62</td><td colspan="2">2.40</td><td colspan="2">2.16</td></tr>
<tr><td>UP2565</td><td colspan="2">2.64</td><td colspan="2">2.47</td><td colspan="2">2.30</td><td colspan="2">2.18</td></tr>
<tr><td>UP2748</td><td colspan="2">2.60</td><td colspan="2">2.50</td><td colspan="2">2.26</td><td colspan="2">2.17</td></tr>
<tr><td>HD3059</td><td colspan="2">2.77</td><td colspan="2">2.65</td><td colspan="2">2.43</td><td colspan="2">2.12</td></tr>
<tr><td></td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.243</td><td colspan="2">0.486</td><td>0.687</td><td>0.159</td><td colspan="2">.0318</td><td>0.450</td></tr>
<tr><td>CD(5%)</td><td>0.700</td><td colspan="2">0.140</td><td>0.198</td><td>0.458</td><td colspan="2">0.917</td><td>0.129</td></tr>
</table>

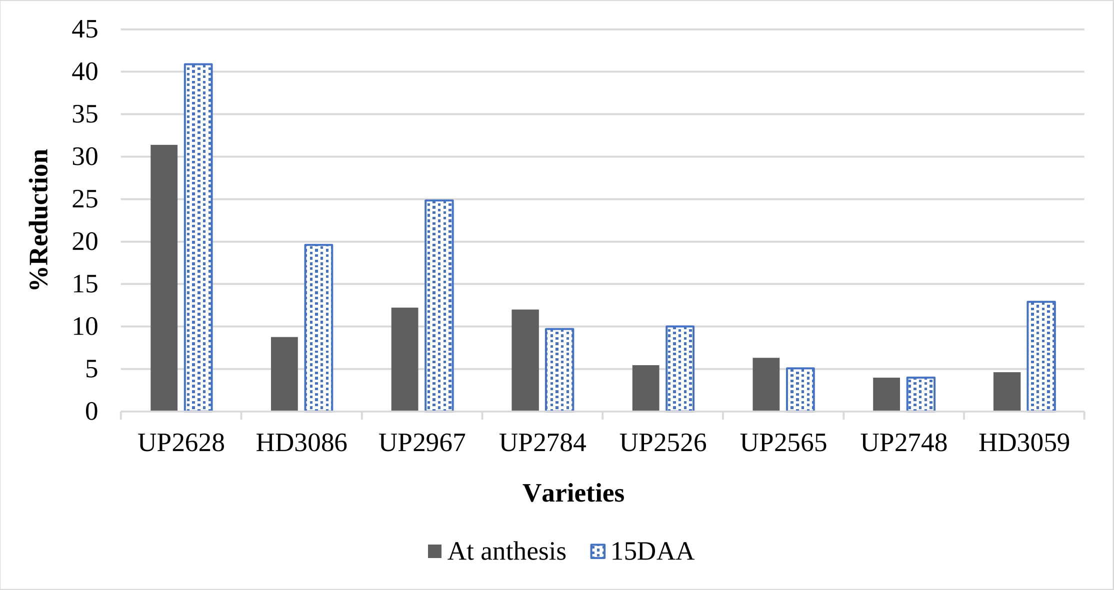


Figure 4.5.1 Percent reduction in total chlorophyll of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.5.2 Chlorophyll 'a'

The chlorophyll 'a' in all wheat varieties was found to be reduced due to D2(elevated temperatures) as compared to D1 conditions. On comparing between the two stages, chlorophyll 'a' in all wheat varieties was found higher at the time of anthesis and tends to reduce as varieties moves towards grain filling stage. In detailed view, at anthesis highest chlorophyll "a" was observed in UP2748 variety (2.50 mg/g) and lowest in HD3086 (1.79 mg/g) under D1 while under D2, total chlorophyll was found to be reduced and highest value observed in UP2748(2.26 mg/g) and lowest in HD3086 variety (1.65 mg/g) (**Table 4.5.2).** Maximum reduction in chlorophyll 'a' due to D2 (elevated temperatures) was recorded in UP2784 (30.06 %) while minimum in UP2565 (6.07 %) (**Figure 4.5.2**). Statistically values of chlorophyll 'a' at anthesis was found significantly affected by elevated temperatures in terms of treatments at ($p \leq 0.01$) while in terms of varieties at ($p \leq 0.05$). Interactions between treatment ×varieties were found non-significant at ($p>0.05$). At grain filling (15 DAA) highest chlorophyll 'a' was observed in UP2628 variety (1.74 mg/g) and lowest in UP2526 (1.17 mg/g) under D1 while under D2, highest value of chlorophyll 'a' was observed in UP2784 and UP2748 (1.41 mg/g) and lowest inUP2565 variety (1.12 mg/g) (**Table**

4.5.2). Maximum reduction in chlorophyll ‘a’ due to D2 (elevated temperatures) was recorded in UP2628 (33.33%) while minimum in UP2526 (1.82%) (**Figure 4.5.2**). Statistically values of chlorophyll ‘a’ at grain filling was significantly affected by D2in terms of treatments and varieties ($p \leq 0.01$). Other interactions between treatments × varieties were also found significant at ($p \leq 0.05$).

Table 4.5.2 Effect of differential temperatures on chlorophyll ‘a’ (mg/g FW) of different wheat varieties. at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="7">Chlorophyll ‘a’ (mg/FW)</th></tr>
<tr><th colspan="4">At anthesis</th><th colspan="3">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th>D2</th><th colspan="2">D1</th><th>D2</th></tr>
<tr><td>UP2628</td><td colspan="2">2.11</td><td>1.75</td><td colspan="2">1.74</td><td>1.16</td></tr>
<tr><td>HD3086</td><td colspan="2">1.79</td><td>1.65</td><td colspan="2">1.48</td><td>1.16</td></tr>
<tr><td>UP2967</td><td colspan="2">2.21</td><td>2.05</td><td colspan="2">1.67</td><td>1.36</td></tr>
<tr><td>UP2784</td><td colspan="2">2.23</td><td>1.56</td><td colspan="2">1.44</td><td>1.41</td></tr>
<tr><td>UP2526</td><td colspan="2">2.31</td><td>2.10</td><td colspan="2">1.17</td><td>1.15</td></tr>
<tr><td>UP2565</td><td colspan="2">2.15</td><td>2.02</td><td colspan="2">1.18</td><td>1.12</td></tr>
<tr><td>UP2748</td><td colspan="2">2.50</td><td>2.26</td><td colspan="2">1.46</td><td>1.41</td></tr>
<tr><td>HD3059</td><td colspan="2">2.11</td><td>1.95</td><td colspan="2">1.65</td><td>1.35</td></tr>
<tr><th></th><th>Treatment (T)</th><th>Variety (V)</th><th>(TXV)</th><th>Treatment (T)</th><th>Variety (V)</th><th>(TXV)</th></tr>
<tr><td>SEm ±</td><td>0.580</td><td>0.116</td><td>0.164</td><td>0.277</td><td>0.555</td><td>0.785</td></tr>
<tr><td>CD(5%)</td><td>0.167</td><td>0.334</td><td>0.473</td><td>0.800</td><td>0.160</td><td>.0226</td></tr>
</table>

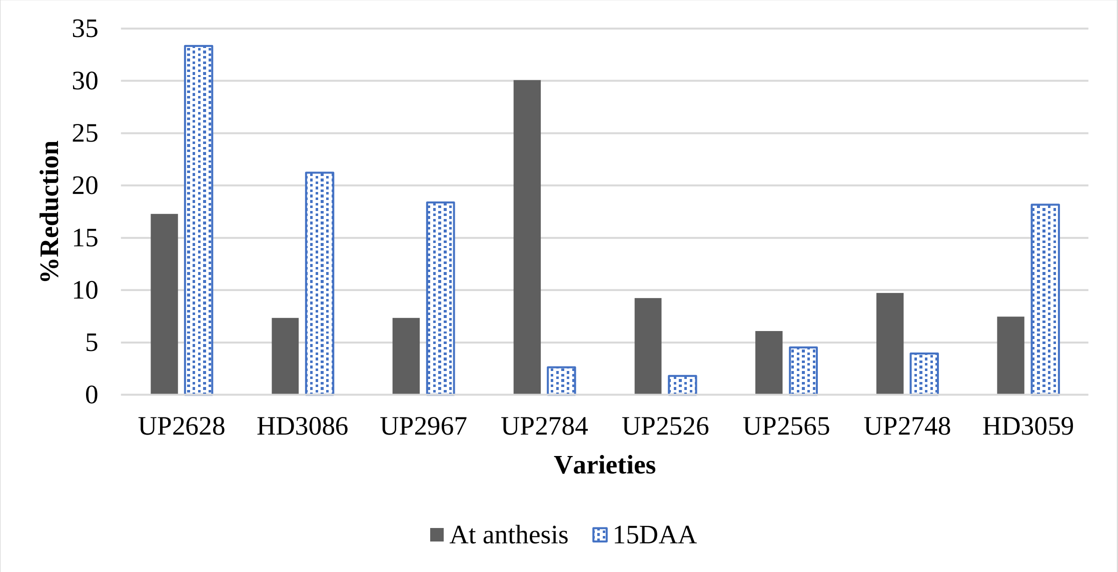


Figure 4.5.2 Percent reduction in chlorophyll ‘a’ of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.5.3 Chlorophyll ‘b’

The Chlorophyll ‘b’ was found to be reduced in all wheat varieties due to D2 (elevated temperatures) as compared to D1 conditions. On comparing between the two stages, chlorophyll ‘b’ in all wheat varieties was found higher at the time of anthesis and tends to reduce as varieties moves towards grain filling stage. In detailed view, at anthesis highest value of chlorophyll “b” was observed in UP2967 variety (0.88 mg/g) and lowest in UP2565 (0.57 mg/g) under D1 while under D2, highest value observed in UP2748 (0.77 mg/g) and lowest in UP2565variety (0.50 mg/g) (**Table 4.5.3).** Maximum reduction in chlorophyll ‘b’ due to D2 (elevated temperatures) was recorded in UP2967 (34.61 %) while minimum in HD3086 and UP2748 (8.54 % and 9.77 % respectively) (**Figure 4.5.3**). Statistically values of chlorophyll ‘b’ at the time of anthesis was significantly affected by D2 (elevated temperatures) in terms of treatments and varieties ($p \leq 0.01$). Other interactions between treatments × varieties were found non-significant at ($p > 0.05$). At grain filling (15 DAA) highest chlorophyll ‘b’ was recorded in UP2967 variety (0.77 mg/g) and lowest in UP2565 (0.47 mg/g) under D1 while under D2, highest value of chlorophyll ‘b’ was observed in UP2784 and HD3059 (0.65 mg/g) and lowest in UP2565 variety (0.42 mg/g) (**Table 4.5.3).** Maximum reduction in chlorophyll ‘b’ due to D2 (elevated temperatures) was recorded in UP2967 (27.32 %) while minimum in UP2748(5.36%) (**Figure 4.5.3**). Statistically, values of chlorophyll ‘b’ at the time of grain filling was significantly affected by D2in terms of treatments and varieties ($p \leq 0.01$). Other interactions between treatments × varieties were found non-significant at ($p > 0.05$).

Table 4.5.3 Effect of differential temperatures on Chlorophyll ‘b’ (mg/g FW) of different wheat varieties. at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="9">Chlorophyll ‘b’ (mg/g FW)</th></tr>
<tr><th></th><th colspan="4">At anthesis</th><th colspan="4">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th colspan="2">D2</th><th colspan="2">D1</th><th colspan="2">D2</th></tr>
<tr><td>UP2628</td><td colspan="2">0.79</td><td colspan="2">0.58</td><td colspan="2">0.68</td><td colspan="2">0.53</td></tr>
<tr><td>HD3086</td><td colspan="2">0.73</td><td colspan="2">0.66</td><td colspan="2">0.62</td><td colspan="2">0.52</td></tr>
<tr><td>UP2967</td><td colspan="2">0.88</td><td colspan="2">0.57</td><td colspan="2">0.77</td><td colspan="2">0.56</td></tr>
<tr><td>UP2784</td><td colspan="2">0.86</td><td colspan="2">0.71</td><td colspan="2">0.72</td><td colspan="2">0.65</td></tr>
<tr><td>UP2526</td><td colspan="2">0.69</td><td colspan="2">0.53</td><td colspan="2">0.55</td><td colspan="2">0.51</td></tr>
<tr><td>UP2565</td><td colspan="2">0.57</td><td colspan="2">0.50</td><td colspan="2">0.47</td><td colspan="2">0.42</td></tr>
<tr><td>UP2748</td><td colspan="2">0.85</td><td colspan="2">0.77</td><td colspan="2">0.52</td><td colspan="2">0.49</td></tr>
<tr><td>HD3059</td><td colspan="2">0.82</td><td colspan="2">0.70</td><td colspan="2">0.71</td><td colspan="2">0.65</td></tr>
<tr><th></th><th>Treatment (T)</th><th colspan="2">Variety (V)</th><th>(TXV)</th><th>Treatment (T)</th><th colspan="2">Variety (V)</th><th>(TXV)</th></tr>
<tr><td>SEm ±</td><td>0.158</td><td colspan="2">0..31</td><td>0.044</td><td>0.158</td><td colspan="2">0.098</td><td>0.107</td></tr>
<tr><td>CD (5%)</td><td>0.457</td><td colspan="2">0.915</td><td>0.129</td><td>0.455</td><td colspan="2">0.316</td><td>0.447</td></tr>
</table>

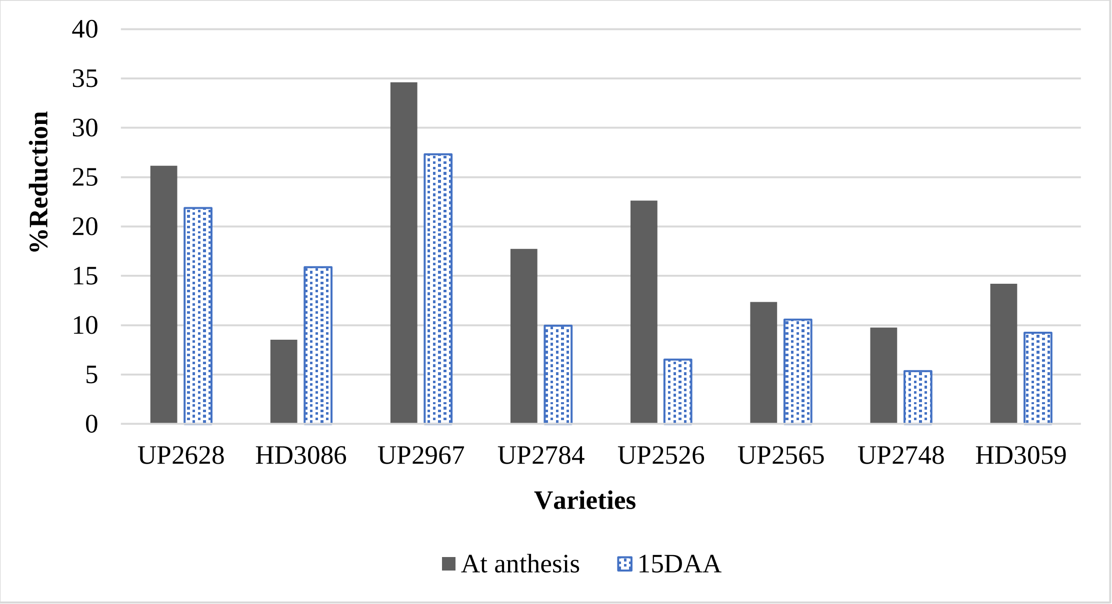


Figure 4.5.3 Percent reduction in chlorophyll ‘b’ of different wheat varieties under D2 conditions in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.5.4. Chlorophyll a/b ratio

The chlorophyll a/b ratio was found to be reduced in all wheat varietiesdue to D2(elevated temperatures) as compared to D1 conditions. On comparing between the two stages, chlorophyll a/b ratio was found higher at the time of anthesis as compared to grain filling stage (15DAA). In detailed view, at anthesis highest chl a/b ratio was observed in UP2565 variety (3.33) and lowest in HD3086 and UP2784 (2.60) under D1 while under D2, highest value of chl a/b ratio observed in UP2565 (3.08) and lowest in UP2784 variety (2.22) (**Table 4.5.4).** Maximum reduction in chl a/b ratio due to D2 (elevated temperatures) was recorded in UP2784 (14.40%) while minimum in UP2748 (2.56%) (**Figure 4.5.4**). Statistically chlorophyll a/b ratio at the time of anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Other interactions between treatments × varieties were found non-significant at ($p > 0.05$). At grain filling (15 DAA) highest chl a/b ratio was recorded in UP2748 variety (2.68) and lowest in UP2526 (2.34) under D1 while under D2, highest value of chl a/b ratio was observed in UP2748 (2.66) and lowest in UP2784 variety (2.16) (**Table 4.5.4).** Maximum reduction in chlorophyll a/b ratio due to D2 (elevated temperatures) was recorded in UP2784 (13.29 %) while minimum reduction in UP2748 (0.60%) (**Figure 4.5.4**). Statistically, chlorophyll a/b ratio at the time of grain filling was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Other interactions between treatments × varieties were found non-significant at ($p > 0.05$).

Table 4.5.4 Effect of differential temperatures on chlorophyll a/b ratio of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="11">Chlorophyll a/b ratio</th></tr>
<tr><th colspan="6">At anthesis</th><th colspan="5">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th colspan="3">D2</th><th colspan="2">D1</th><th colspan="3">D2</th></tr>
<tr><td>**UP2628**</td><td colspan="2">2.68</td><td colspan="3">2.31</td><td colspan="2">2.58</td><td colspan="3">2.27</td></tr>
<tr><td>**HD3086**</td><td colspan="2">2.60</td><td colspan="3">2.44</td><td colspan="2">2.38</td><td colspan="3">2.24</td></tr>
<tr><td>**UP2967**</td><td colspan="2">2.69</td><td colspan="3">2.45</td><td colspan="2">2.43</td><td colspan="3">2.26</td></tr>
<tr><td>**UP2784**</td><td colspan="2">2.60</td><td colspan="3">2.22</td><td colspan="2">2.49</td><td colspan="3">2.16</td></tr>
<tr><td>**UP2526**</td><td colspan="2">2.75</td><td colspan="3">2.60</td><td colspan="2">2.34</td><td colspan="3">2.24</td></tr>
<tr><td>**UP2565**</td><td colspan="2">3.33</td><td colspan="3">3.08</td><td colspan="2">2.50</td><td colspan="3">2.44</td></tr>
<tr><td>**UP2748**</td><td colspan="2">3.02</td><td colspan="3">2.94</td><td colspan="2">2.68</td><td colspan="3">2.66</td></tr>
<tr><td>**HD3059**</td><td colspan="2">2.77</td><td colspan="3">2.59</td><td colspan="2">2.54</td><td colspan="3">2.26</td></tr>
<tr><td></td><td>**Treatment (T)**</td><td colspan="2">**Variety (V)**</td><td colspan="2">**(TXV)**</td><td>**Treatment (T)**</td><td colspan="2">**Variety (V)**</td><td colspan="2">**(TXV)**</td></tr>
<tr><td>**SEm ±**</td><td>0.480</td><td colspan="2">0.961</td><td colspan="2">0.135</td><td>0.292</td><td colspan="2">0.584</td><td colspan="2">0.826</td></tr>
<tr><td>**CD (5%)**</td><td>0.138</td><td colspan="2">0.276</td><td colspan="2">0.391</td><td>0.841</td><td colspan="2">0.168</td><td colspan="2">0.238</td></tr>
</table>

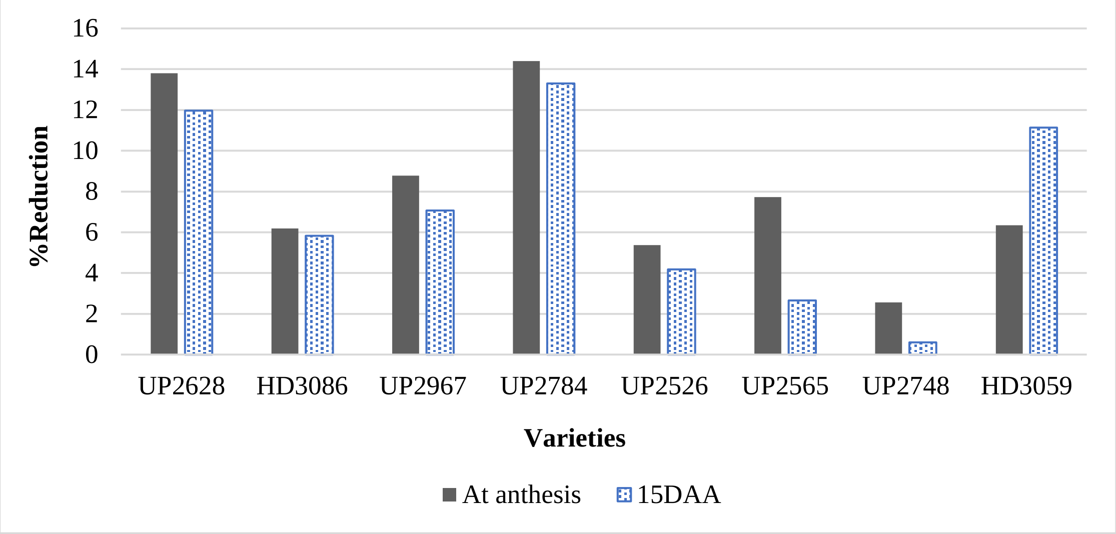


Figure 4.5.4 Percent reduction in chlorophyll a/b ratio of different wheat varieties under D2 conditions in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present research, total chlorophyll, chlorophyll ‘a’ &‘b’ and chlorophyll a/b ratio in flag leaves of each wheat varieties were observed under both sowing conditions at anthesis and grain filling stage. The results showed that all the chlorophyll parameters were found reduced due to elevation in temperature under D2 as compared to D1 as well as these photosynthetic pigments were found higher during anthesis and reduced 15 days after anthesis. From past researches, as a result of elevation in temperatures in winter crops like wheat causes heat stress during their growth period responsible for disruption in photosynthesis process due to disruption in structure and function of chloroplast as reduction in chlorophyll content and amount of its pigments observed (**Xu *et al*., 1995**). In previous researches reduced trend was reported in chlorophyll content, chlorophyll pigments (a and b) and ratio of pigments in wheat sown under elevated temperatures condition as compared to normal conditions. The excess light and oxidative stress causes inactivation of chloroplast enzyme, protein degradation and membrane rupture under elevated temperature and decreased total chlorophyll and chlorophyll pigments hence rate of leaf photosynthesis decreased (**Sairam *et al*., 2000**). An experiment was done with different wheat varieties under two sowing conditions i.e. 27th November2003 and 20thDecember2003. The total chlorophyll and chlorophyll a/b ratio was found reduced under December sowing as compared to November sowing conditions. A loss of 25 to 30 % in total chlorophyll and chlorophyll a to b ratio was reported when varieties move towards grain filling stage when compared with anthesis stage. This decline in total chlorophyll and chlorophyll a/b ratio may be due to the loss of chlorophyll ‘a’ containing protein under heat stress condition which are closely associated with PSII and PSI reaction centres **(Srivastava, 2005)**. According to previous studies the reduction in chlorophyll content in wheat leaves was due to heat induced damage to thylakoids. It was observed that protochlorophyllide oxidoreductase (POR) was greatly reduced under heat stressed wheat cultivars as compared to control shows a significant positive correlation between loss of chlorophyll and heat stress. In a similar study in which an experiment was done. Higher values of total chlorophyll and chlorophyll a/b ratio were observed at the time of anthesis and after 10, 20 and 30 days after anthesis, the total chlorophyll and chlorophyll a/b ratio were found reduced in all wheat varieties. Under optimum sowing condition, maximum total chlorophyll was observed in VL802 (4.620 mg/g) and minimum observed in PBW 373 (3.513 mg/g) while under elevated heat stress conditions, total chlorophyll was reduced and maximum observed in VL738 (3.47 mg/g) and minimum in PBW 396 (2.956 mg/g). Similarly, for chl a/b ratio, under optimum sowing, maximum value observed in VL738 (3.90) and minimum in PBW373 (2.87) while under elevated temperatures maximum value observed in VL738 (3.10) and minimum in PBW373 (2.00). Such reduction under elevated temperatures due to plant experiences heat stress during anthesis and after anthesis resulting onset of early senescence and early yellowing of the flag leaves hence total chlorophyll and chl a/b found reduced under stress condition (**Chandra, 2006)**. In another experiment chlorophyll content of flag leaves in wheat decreased after anthesis and least chlorophyll content observed during grain filling stage (**Cai *et al.*, 2008**). In order to check the effect of sowing condition on total chlorophyll content, chlorophyll ‘a’ and chlorophyll ‘b’ in leaves of wheat, 36 different genotypes were analysed under two sowing condition heat stressed and normal conditions. During anthesis mean leaf chlorophyll (SPAD value) under normal and heat stressed condition was same. While after 15 days of anthesis, mean leaf chlorophyll under normal conditions was 34.18 mg/g and under heat stressed it was reduced to 33.34 mg/g. Chlorophyll a and b was found reduced under elevated temperatures as compared to non-stressed condition in all wheat genotypes. A reduction up to 24.80% in chl ‘a’ at anthesis and 9.70% at grain filling (15 DAA) was observed under heat stressed as compared to normal sowing conditions. Similarly, a reduction upto 35.99 % in chl ‘b’ at anthesis and 64.97 % at grain filling (15 DAA) was observed under heat stressed condition as compared to normal sowing conditions (**Dhyani, 2010).** In another research, six different wheat cultivars (UP-2338, PBW-343, UP-2113, PBW-175, VL-616 and VL-421) were subjected to variable temperature conditions by a delay of 20 days in their sowing. A reduction in total chlorophyll and chl a/b ratio was observed under late sowing as compared to normal sown cultivars. The highest value (4.1 mg^{-1}g fw) of total chlorophyll during anthesis and chl a/b ratio (3.8) was observed in UP-2338 under normal sown conditions which declined to 1.5 and1.6 respectively under late sown condition. According to author, declination in chlorophyll pigments and total chlorophyll is one of the most important characteristic features of onset of senescence. Delay in sowing induces senescence quickly than the normal sown conditions, may be due to imposition of adverse environmental variables over late sown cultivars (**Srivastava *et al*., 2012)**. To study the effect of elevated temperatures on chlorophyll content,12 different soybean genotypes were grown at day/night temperatures of 30/22, 34/24, 38/26 and 42/28 °C with an average temperature of 26, 29, 32 and 35 °C, respectively, under greenhouse conditions. The results showed that the chlorophyll content was significantly influenced by increasing temperature and a reduction of 5%, 8% and 11% were observed in the plants grown at 30/22, 34/24 and 38/26 °C, while maximum reduction of 21% observed at 42/28 °C as compared to ambient temperature conditions, respectively (**Jumrani *et al*., 2017).** The elevated temperatures treatments up to 37ºC and 45ºC for 8 hours on seedings of Karacadage and Firat wheat cultivars showed induced reduction in chlorophyll accumulation under heat stress condition (**Iqbal *et al*., 2017**). An experiment conducted with 7 wheat varieties (Joe, SY Monument, Larry,West Bred 4458, Zenda, West Bred Cedarand Everest to study the effect of higher temperatures on chlorophyll pigments under heat stress (35/15 ◦C) and normal 25/15 ◦C condition. The results showed that as the varieties moves towards senescence, a steep declination in chlorophyll and chlorophyll index observed in flag leaves for all varieties and treatments, but the declination was fast in those wheat varieties which are under heat stress exposure (**Sebal *et al.*, 2020).** According to the recent studies it is also now well-known that thermo-tolerant genotypes possess higher chlorophyll or lower reduction in chlorophyll content under elevated temperatures as compared to ambient. For this purpose, two spring wheat cultivars (Millet-11, Punjab-11) and two advanced lines (V-07096, V-10110) were exposed to terminal heat stress under late sown condition. The results showed that the wheat crops expressed a 20% and 71–125% increase in Chl a and b contents respectively, under late sown compared to normal sown crop during anthesis. Higher leaf chlorophyll contents at anthesis in wheat genotypes are an indication of delayed senescence, high photosynthetic rate and remobilization of assimilates under terminal heat stress (**Tariq *et al.*, 2021).**

4.5.5 Carotenoid content

The carotenoid content was found to be reduced under D2 (elevated temperatures) as compared to D1 conditions. On comparing between the two stages, carotenoid in all wheat varieties was found higher at the time of anthesis and tends to reduce as varieties moves towards grain filling stage. In detailed view, at anthesis highest value of carotenoids was observed inHD3059 variety (0.59 mg/g) and lowest in UP2526(0.37 mg/g) under D1while under D2, highest value observed in HD3059 (0.51 mg/g) and lowest in UP2628 variety (0.25 mg/g) (**Table 4.5.5).** Maximum reduction in carotenoid content due to D2 (elevated temperatures was recorded in UP2628 (49.08%) while minimum in UP2565 (4.70 %) (**Figure 4.5.5**). Statistically values of carotenoids at the time of anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interactions between treatments × varieties were also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest carotenoid was recorded in HD3059 variety (0.56 mg/g) and lowest in UP2526 and UP2565 (0.35 mg/g) under D1 while under D2, highest value of carotenoid was observed in HD3059 (0.48 mg/g) and lowest in UP2628 variety (0.23 mg/g) (**Table 4.5.5).** Maximum reduction in carotenoid content due to D2 (elevated temperatures) was recorded in UP2628 (51.96%) while minimum in UP2748 (4.48%)

(**Figure 4.5.5**). Statistically values of carotenoid at the time of grain filling was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interactions between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.5.5 Effect of differential temperatures on carotenoid content (mg/g FW) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="7">Carotenoid content (mg/g FW)</th></tr>
<tr><th colspan="4">At anthesis</th><th colspan="3">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th>D2</th><th colspan="2">D1</th><th>D2</th></tr>
<tr><td>UP2628</td><td colspan="2">0.50</td><td>0.25</td><td colspan="2">0.48</td><td>0.23</td></tr>
<tr><td>HD3086</td><td colspan="2">0.51</td><td>0.47</td><td colspan="2">0.44</td><td>0.38</td></tr>
<tr><td>UP2967</td><td colspan="2">0.46</td><td>0.42</td><td colspan="2">0.45</td><td>0.38</td></tr>
<tr><td>UP2784</td><td colspan="2">0.43</td><td>0.36</td><td colspan="2">0.38</td><td>0.35</td></tr>
<tr><td>UP2526</td><td colspan="2">0.37</td><td>0.34</td><td colspan="2">0.35</td><td>0.32</td></tr>
<tr><td>UP2565</td><td colspan="2">0.43</td><td>0.41</td><td colspan="2">0.35</td><td>0.32</td></tr>
<tr><td>UP2748</td><td colspan="2">0.55</td><td>0.49</td><td colspan="2">0.42</td><td>0.40</td></tr>
<tr><td>HD3059</td><td colspan="2">0.59</td><td>0.51</td><td colspan="2">0.56</td><td>0.48</td></tr>
<tr><td></td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.947</td><td>0.189</td><td>0.268</td><td>0.885</td><td>0.177</td><td>0.250</td></tr>
<tr><td>CD(5%)</td><td>0.273</td><td>0.546</td><td>0.772</td><td>0.255</td><td>0.510</td><td>0.721</td></tr>
</table>

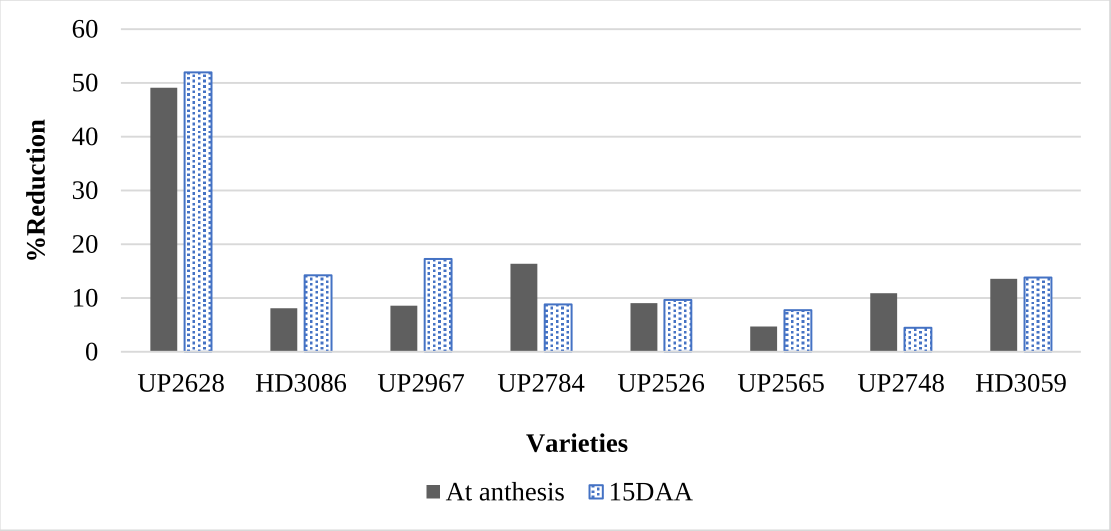


Figure 4.5.5 Percent reduction in carotenoid content of different wheat varieties under D2 conditions in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In the present study carotenoids was found reduced under D2 as compared to D1. On comparing between the two heat sensitive stages of wheat life cycle the value of carotenoids was found higher at the time of anthesis and after anthesis it was found reduced. The carotenoids are the yellow pigment mainly derived from Xanthophylls considered as minor constituents in cereal (**Kruger and Reed, 1988**). Some wheat carotenoids such as α-carotene and β-cryptoxanthin have provitamin A activity. The carotenoids which do not have vitamin activity, contribute to antioxidant activity such as; Lutein, β-cryptoxanthin, zeaxanthin and β-carotene are carotenoids which act as antioxidants and found in wheat. All carotenoids can easily oxidise, and thus they are readily lost during milling and heat processing. (**Van den Berg *et al*., 2000**). From previous findings it was concluded that heat tolerant cultivar of wheat (Fang) had high chlorophyll content as well as carotenoids as compared to heat sensitive cultivar (Siete Cerros) (**Tahir *et al*., 2009**). Other studies followed the general trend of reduced carotenoid content under elevated temperatures, such as in an experiment wheat variety (*Trtiicum aestivu*m L., cv. Shahryar) was exposed under two different conditions: under controlled environmental conditions with day/night temperatures of 25/20°C (control) and under treatment of 30/25°C and 35/30°C. The results showed that the carotenoids contents found decreased significantly at high temperature stress (**Amirjani, 2012).** The effect of heat stress on carotenoid content of 11 wheat genotypes was evaluated and found decreased carotenoid content (within range of 0.0291-0.298 mg/g FW) in all the genotypes under heat stress as compared to control (**Kaur and Thid, 2017**). Similar trend was found when an experiment was performed with 10 different wheat genotypes under controlled condition (day-night temperature (30±9°C and 13±7°C) and heat treatment (in internal glasshouse temperature which was maintained at 35- 40°C for 7 days during anthesis for 5 hours). The results showed that high temperature, causes a decline from 47.17 to 73.99 % in carotenoids content in the majority of genotypes (**Khan *et al*., 2020**). In a study, four wheat varieties having difference in heat stress tolerance i.e. a high yielding, and heat tolerant: BARI Gom 26 (BG26), moderately heat tolerant: BARI Gom 25 (BG25) &BARI Gom 23 (BG23) and highly heat susceptible variety: Pavon 76 were evaluated for physiological and biochemical responses and exposed to 25º C (control) and 35 ºC (heat stress) condition. The results concluded that there was a decrease in carotenoid content in stressed condition compared to non-stress condition. They also conclude that carotenoid content in the wheat leaves decreased markedly due to heat stress in all varieties but the decrease was not statistically significant. Maximum reduction in carotenoid content was observed in Pavon 76 (40%) and minimum reduction observed in BG26 (11.76 %). According to the author, carotenoids help in protecting photosystems and chlorophyll molecules by reacting with lipid peroxidation products and scavenging singlet oxygen. High stability of carotenoids under heat stress condition indicates enhanced defence system in tolerant wheat variety “BG26” compared to other varieties. This could be the reason for slower degradation of carotenoids allowing stability in the light absorption machinery in “BG26” under heat stress. (**Mohi-Ud-Din *et al.*, 2021)**. Six wheat (*Triticum aestivum*L.) cultivars, i.e Millat-2011, Galaxy-2013, Fsd-2008, Sahar-2006, Lasani-2008 and Shafaq-2006 were exposed to under two conditions in a glass chamber 25ºC (controlled) and heat stress (37 °C) daily for 10 h (treatment). A gradual decrease in carotenoid content under heat stress was reported. Least reduction in carotenoids content was observed in Galaxy-2013 and Millat-2011 while the maximum reduction in Shafaq-2006. The author concludes that the accumulation of carotenoids could be helpful to protect different cellular structures from ROS damage leading to membrane stability under stressful environments (**Gulnaz *et al.*, 2021**).Overexpression of *IbOr-R96H* in transgenic sweet potato leads to production of higher carotenoid content under abiotic stress condition which provide greater tolerance to heat stress (47 °C).During this experiment, the total carotenoid contents were found 5.4–19.6 fold higher in transgenic than non-transgenic sweet potato. **(Kim *et al.*, 2021).**

4.5.6 Proline Content

In present study, proline content of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling). Proline act as an osmo-protectant and considered as metabolic marker for identification of thermo-tolerant genotype. The results showed that the proline was found to be increased under D2 (elevated temperatures) as compared to D1. On comparing between the two sensitive stages, proline in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage. In detailed view, at anthesis highest proline content was observed in UP2748 variety (10.27 µ M) and lowest in

UP2628 (5.11 µ M) under D1 while under D2, highest value observed in UP2748 (11.87 µ M) and lowest in UP2628 variety (8.36 µ M) (**Table 4.5.6).** Maximum increment in proline content due to D2 (elevated temperature) was recorded in UP2784 (65.56 %) while minimum in HD3086 (1.80 %) (**Figure 4.5.6**). Statistically proline content at the time of anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were found non-significant at ($p > 0.05$). At grain filling (15 DAA) highest proline was recorded in UP2526 variety (12.82 µ M) and lowest in UP2784 (7.05 µ M) under D1 while under D2 highest value of proline was observed in UP2748 (16.68 µ M) and lowest in UP2784 variety (11.27 µ M) (**Table 4.5.6).** Maximum increment in proline due to D2 (elevated temperatures) was recorded in UP2565 (75.05 %) while minimum in HD3086 (3.79 %) (**Figure 4.5.6**). Statistically proline at grain filling was significantly affected by elevation in temperatures terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). In present research, proline content was found increased under elevated temperatures as compared to non-stress conditions along with higher proline observed under grain filling stage as compared to anthesis.

Table 4.5.6 Effect of differential temperatures on proline content (µ M per g tissue) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="13">Proline content (µ M per g tissue)</th></tr>
<tr><th colspan="7">At anthesis</th><th colspan="6">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="3">D1</th><th colspan="3">D2</th><th colspan="3">D1</th><th colspan="3">D2</th></tr>
<tr><td>UP2628</td><td colspan="3">5.11</td><td colspan="3">8.36</td><td colspan="3">7.68</td><td colspan="3">12.00</td></tr>
<tr><td>HD3086</td><td colspan="3">8.65</td><td colspan="3">8.80</td><td colspan="3">11.26</td><td colspan="3">11.69</td></tr>
<tr><td>UP2967</td><td colspan="3">7.91</td><td colspan="3">9.04</td><td colspan="3">9.21</td><td colspan="3">12.70</td></tr>
<tr><td>UP2784</td><td colspan="3">6.16</td><td colspan="3">10.19</td><td colspan="3">7.05</td><td colspan="3">11.27</td></tr>
<tr><td>UP2526</td><td colspan="3">8.10</td><td colspan="3">11.48</td><td colspan="3">12.82</td><td colspan="3">14.93</td></tr>
<tr><td>UP2565</td><td colspan="3">7.93</td><td colspan="3">10.95</td><td colspan="3">8.20</td><td colspan="3">14.35</td></tr>
<tr><td>UP2748</td><td colspan="3">10.27</td><td colspan="3">11.87</td><td colspan="3">11.91</td><td colspan="3">16.68</td></tr>
<tr><td>HD3059</td><td colspan="3">8.73</td><td colspan="3">11.16</td><td colspan="3">10.27</td><td colspan="3">14.76</td></tr>
<tr><td></td><td colspan="2">Treatment (T)</td><td colspan="2">Variety (V)</td><td colspan="2">(TXV)</td><td colspan="2">Treatment (T)</td><td colspan="2">Variety (V)</td><td colspan="2">(TXV)</td></tr>
<tr><td>SEm ±</td><td colspan="2">0.233</td><td colspan="2">0.466</td><td colspan="2">0.659</td><td colspan="2">0.178</td><td colspan="2">0.356</td><td colspan="2">0.504</td></tr>
<tr><td>CD(5%)</td><td colspan="2">0.671</td><td colspan="2">1.343</td><td colspan="2">1.900</td><td colspan="2">0.513</td><td colspan="2">1.026</td><td colspan="2">1.451</td></tr>
</table>

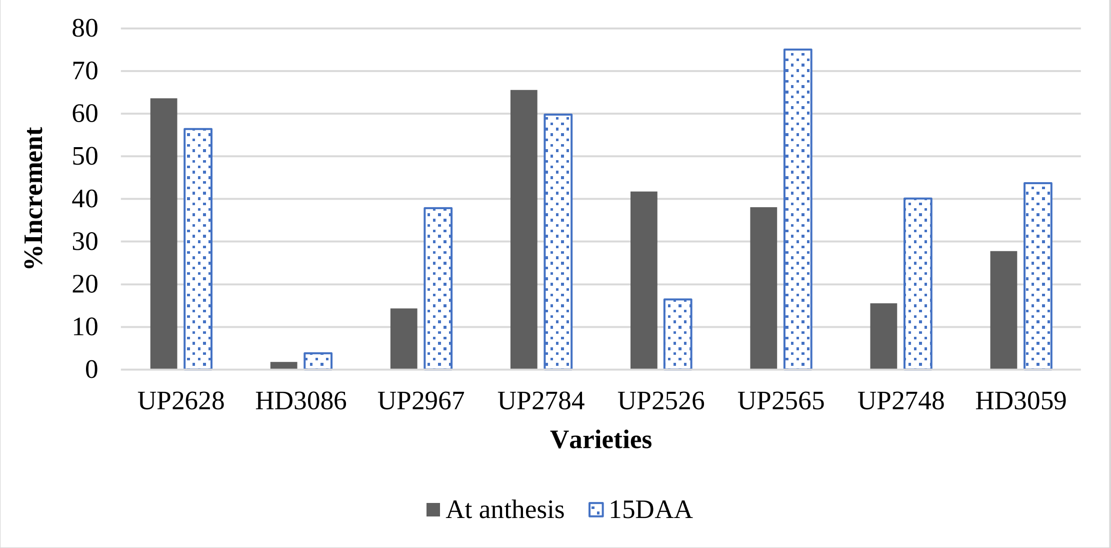


Figure 4.5.6 Percent increment in proline content of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

From previous literature, it was found that in response to environmental stresses, accumulation of proline taken place in leaves which act as compatible solutes and helps in maintaining osmotic concentrations in cells (**Arshi *et al*., 2005**). During an experiment, proline content was found increased with the growth stages rom vegetative stage to anthesis and from anthesis to 15 days after anthesis) in different wheat genotypes. Accumulation of proline is an adaptive mechanism from which plants cope up with various abiotic stresses. Under elevated temperatures plants experiences heat stress as well as water stress which causes activation of biosynthesis of proline and inactivation of degradation of proline which results accumulation of proline in flag leaves (**Dhyani, 2010**). A wheat variety (shahryar) was exposed under controlled environmental conditions with day/night temperatures of 25/20 °C (control) and elevated temperatures 30/25 °C and 35/30 °C. The results showed that the proline content increased significantly at high temperature stress. The proline content under controlled condition was observed (0.98 ± 016 µ M) while under first treatment (30/25 °C) it was increased to (1.38 ± 0.16 µ M) and under second treatment (35/30 °C) it was further increased to (1.97 ± 0.17 µ M). As plant moves towards maturity, elevation in air temperature also increases which lead to increase in accumulation of more proline during grain filling in comparison to anthesis stage

(Iqbal *et al.,* 2017). Flag leaves of four different wheat genotypes was analysed at 18 days after anthesis (grain filling stage) under normal, moderate and high temperature conditions. Under normal condition, all 4 wheat genotypes showed proline content within range between (1.00 to 1.21 µ M per g FW) in flag leaf. However, with the elevation in temperature, the proline was found increased. Under moderate temperature, the proline content was found within the range of (1.26 µ M per g FW to 2.09 µ M per g FW). Under high temperature, proline content was found in the range of (1.78 µ per g FW to 2.77 µ per g FW) (**Sharmin *et al.,* 2020**). In a recent experiment a significant increase in proline content was observed under heat stress as compared to control. Results indicate that proline content in flag leaves of wheat variety Faisalabad-2008, under no stress/controlled condition (well-watered and normal condition) was 3.86 ± 0.46 µ per g FW while under heat stress (inside the plastic tunnel) it was increased to 9.45 ± 0.58 µ per g FW (**Sattar *et al.,* 2020**). In another investigation, 17 wheat genotypes were sown under two sowing conditions with interval of 30 days. The results showed that high temperature stress cause accumulation of more proline content in flag leaved of wheat genotypes; NIA-Sunder, NIA-AS-14-10 and SRN-09111. Under non-stress condition (normal sowing) proline content varied between 3-10 µ M. while, under late sowing it raised by 4 to 5 times, such increment in proline accumulation due to increase in γ-glutamyl kinase and decrease in proline oxidase activities while in heat sensitive genotypes they failed to accumulate more proline as compared to tolerant genotypes **(Khan *et al.,* 2020).** Similar experiment was done with 20 wheat genotypes advance lines (mutants) under two different sowing dates i.e November 15 as normal sowing and December 25 as late sowing. Proline content was found to be increased under December sowing as compared to normal sowing. Under normal sowing proline content was found within range from 6.0 µ per g FW to 19.3 µ per g FW whereas it was significantly increased many folds under stress condition due December sowing ranged between from 49.0 µ per g FW to 350 µ per g FW. Tolerant wheat genotypes showed more accumulation of proline than susceptible genotypes. Author also concludes that the elevated levels of proline with elevated temperatures showed that the genotypes are better adapted to stress environments (**Laghari *et al.,* 2021**). A study in which four wheat varieties having difference in heat stress tolerance i.e. a high yielding, and heat tolerant: BARI Gom 26 (BG26), moderately heat tolerant: BARI Gom 25 (BG25) & BARI Gom 23 (BG23) and highly heat susceptible variety: Pavon 76 were evaluated for physiological and biochemical responses when they were exposed to 25º C (control) and 35 ºC (heat stress) condition. The results conclude that there was increase in proline content in stressed condition compared to non-stress condition. Proline content increased by 17 % in BG23, 25 % in BG25, 39 % in BG26 and 12 % in Pavon 76 compared to control. The results also conclude that maximum percentage of proline accumulated in heat tolerant Variety and least in susceptible (**Mohi-Ud-Din *et al*., 2021)**.

4.5.7 Nitrate Reductase activity

Nitrate reductase (NR) activity of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling). NR activity was found reduced under D2 (elevated temperatures) as compared to D1 conditions. On comparing between the two stages, NR activity in all wheat varieties was found higher at the time of anthesis and tends to reduce as varieties moves towards grain filling stage. In detailed view, at anthesis highest NR activity was observed in UP2628 variety (1.73 µ M) and lowest in HD3059 (0.90 µ M) under D1 while under D2 highest value observed in UP2628(1.27 µ M) and lowest in UP2784 variety (0.57 µ M) (**Table 4.5.7).** Maximum reduction in NR activity due to D2 was recorded in UP2784 (31.31 %) while minimum in UP2526 (10.24 %) (**Figure 4.5.7**). Statistically NR activity at anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest NR activity was recorded in UP2748 variety (0.60 µ M) and lowest in HD3059 (0.27 µ M) under D1 while under D2 highest value NR activity was observed in UP2748 (0.54 µ M) and lowest in HD3059 variety (0.20 µ M) (**Table 4.5.7).** Maximum reduction in NR activity due to D2 (elevation in temperatures was recorded in UP2784 (75.75 %) while minimum in UP2748 (10.62 %) (**Figure 4.5.7**). Statistically, NR activity at grain filling was significantly affected by D2in terms of treatments and varieties ($p \leq 0.01$) while interaction between treatments × varieties were found non-significant at ($p > 0.05$).

Table 4.5.7 Effect of differential temperatures on Nitrate Reductase activity (µ M NO^{2-}/g FW h^{-1}) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="7">Nitrate Reductase activity (µ M NO^{2-}/g FW h^{-1})</th></tr>
<tr><th></th><th colspan="3">At anthesis</th><th colspan="3">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th>D2</th><th colspan="2">D1</th><th>D2</th></tr>
<tr><td>UP2628</td><td colspan="2">1.73</td><td>1.27</td><td colspan="2">0.59</td><td>0.45</td></tr>
<tr><td>HD3086</td><td colspan="2">0.93</td><td>0.70</td><td colspan="2">0.54</td><td>0.29</td></tr>
<tr><td>UP2967</td><td colspan="2">0.91</td><td>0.71</td><td colspan="2">0.47</td><td>0.22</td></tr>
<tr><td>UP2784</td><td colspan="2">0.83</td><td>0.57</td><td colspan="2">0.36</td><td>0.28</td></tr>
<tr><td>UP2526</td><td colspan="2">1.24</td><td>1.11</td><td colspan="2">0.41</td><td>0.30</td></tr>
<tr><td>UP2565</td><td colspan="2">1.30</td><td>0.94</td><td colspan="2">0.44</td><td>0.34</td></tr>
<tr><td>UP2748</td><td colspan="2">1.25</td><td>0.99</td><td colspan="2">0.60</td><td>0.54</td></tr>
<tr><td>HD3059</td><td colspan="2">0.90</td><td>0.74</td><td colspan="2">0.27</td><td>0.20</td></tr>
<tr><td></td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.821</td><td>0.164</td><td>0.232</td><td>0.144</td><td>0.288</td><td>0.407</td></tr>
<tr><td>CD(5%)</td><td>0.236</td><td>0.473</td><td>0.669</td><td>0.414</td><td>0.829</td><td>0.117</td></tr>
</table>

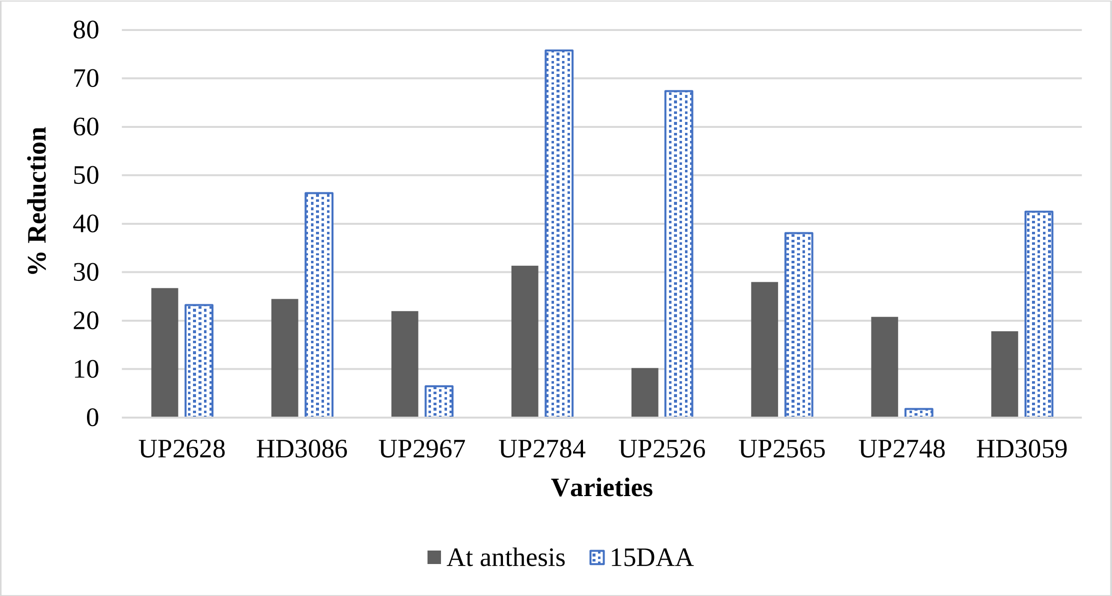


Figure 4.5.7 Percent reduction in NR activity of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively)*

In present study, nitrate reductase activity was found reduced under D2 as compared to D1. NR activity was also found higher at the time of anthesis and reduced as wheat varieties moves towards grain filling. According to previous reports, the nitrate assimilation in higher plants occurs through reduction of nitrate to ammonia in two sequential steps which includes reduction of nitrate to nitrite which is catalysed by an enzyme called nitrate reductase (NR) and reduction of nitrite to ammonia which is catalysed by another enzyme called nitrite reductase (NiR). In wheat, different genotypes showed different ability to accumulate the reduced nitrogen for their growth and development (**Eilrich and Hageman, 1973)**. In previous researches, NR activity found reduced due to elevation in temperatures as compared to normal sowing (no heat stress) conditions in wheat. For example, in one research, 12 different wheat genotypes were analysed under two sowing condition 20 November and 23 December in order to check the effect of sowing condition on NR activity in leaves of wheat at the time of anthesis and 15 days after anthesis. The results showed that there was significant difference in values of NR activity under December sowing as compared to November sowing as well as NR activity in all wheat genotypes was found reduced as the genotypes moves towards grain filling stage (15 DAA). During anthesis lowest and highest NR activity was found within the range of 0.62 µ M to 2.73 µ M under November sowing while under December sowing, the range was reduced and found between 0.48 µ M to 1.77 µ M. Similarly, during grain filling lowest and highest NR activity was found within the range of 0.218 µ M to 1.530 µ M under November sowing while under December sowing it was reduced and become within range of 0.217 to 0.787 µ M (**Dhyani *et al*., 2010).** Similar study in which nitrate reductase activity was found decreased under elevated temperatures also observed in some other crop species such as when sunflower plants (*Helianthus annuus* L) were subjected to temperature stress by exposing plants to 35 or 45 °C for 12 hour there was reduced nitrate reductase activity observed. The higher the temperature (45 °C) more deleterious and decreased NR activity was recorded. At 45 °C NR activity was reduced by 19.38% in comparison to control (27 ºC) no heat stress, while at 35 °C, NR activity was found decreased by 7.74 %. According to the author, the reduction in nitrate reductase activity in plants under heat stress is a biochemical adaptation of plants to conserve energy by stopping nitrate assimilation during stressful condition **(Akladious, 2014**). In *Solanum lycopersicum* (tomato) the effects of heat stress on root nutrient (N) uptake at 25 ◦C/20 ◦C (day/night), 35 °C /30 °C (moderate heat) and 42 °C/37 °C (severe heat) were evaluated. The results showed that the roots were found more affected than shoots which decreases the root:shoot mass ratio, shoot vs. root percentage of nitrogen and carbon, and levels of nutrient-uptake and assimilation proteins in roots led to decrease in nitrogen assimilatory proteins; nitrate reductase and nitrite reductase **(Giri *et al*., 2017).** An experiment was performed to investigate the effect of high temperatures on wheat *(T.aestivium*) cultivars and it was found that NR activity was maximum during pre -flowering stage than post flowering in wheat cultivar. NR activity found higher in tolerant (C-306) than heat sensitive (HUW-468) (**Kumari and Hemantaranjan, 2019**). A study was done in two heat-tolerant (DK-6789; NT-6621) and two heat sensitive (FH-988 and FH-1137) maize genotypes to study the effect of heat stress (40 °C for 2h, 4h and 6h) and controlled (no heat stress provided) conditions on their biochemical performances. The results showed that biochemical attributes such as total soluble proteins (TSP) and nitrate reductase activity were found decreased under heat stress as compared to their controls. Nitrate reductase activity (NR) was found significantly reduced under stress condition. Maximum NR activity was recorded in tolerant genotype DK-6789 and NT-6621 for all the stress treatments, while minimum was observed in FH-988 and FH-1137. According to the author, high temperatures cause increase in the kinetic energy of molecules across the membrane which results membrane loosing and protein denaturation (such ribulose bisphosphate carboxylase and nitrate reductase activity) which affects the overall nitrogen assimilation by decrease the conversion of nitrate to nitrite hence NR activity found decreased under stress condition **(Ayub *et al*., 2020).** The effect of elevated temperature on nitrate reductase activity in *Ulva prolifera* (green algae) was observed under five different temperatures (10, 15, 20, 25 and 30 °C) for 7 days. The results shows that the maximum nitrate reductase activity observed at low temperature range (10, 15, and 20

°C) while the algal growth was found decreased with decrease in its NR activity at higher temperatures (25 and 30 °C). Form the it is clear that nitrate reductase activity is a suitable marker for evaluation of the effects of temperatures on grow, uptake and metabolization of nitrogen nutrients (**Feng *et al*., 2021).**

4.6 Effect of elevated temperatures on lipid peroxidation and antioxidant activity.

4.6.1 Malondialdehyde Assay (MDA)

In present study, MDA content of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling). Malondialdehyde assay **(MDA)** considered as marker for oxidative lipid injury caused by heat stress. The results showed that the MDA content was found to be increased under D2 (elevated temperatures) as compared to D1. On comparing between the two stages, MDA in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage. In detailed view, at anthesis highest MDA content was observed in HD3059 variety (40.34 µ M) and lowest in UP2565 (20.21 µ M) under D1 while under D2, highest value observed in UP2628 (49.57 µ M) and lowest in UP2565 variety (27.00 µ M) (**Table 4.6.1).** Maximum increment in MDA content due to D2 (elevated temperatures) was recorded in UP2628 (79.66 %) while minimum in HD3059 (11.54 %) (**Figure 4.6.1**). Statistically, values of MDA content at anthesis were significantly affected by sowing timing in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest MDA was recorded in UP2628 variety (43.01 µ M) and lowest in UP2784 (40.28 µ M) under D1 while under D2 in UP2748 variety (44.77 µ M) (**Table 4.6.1).** Maximum increment in MDA due to D2 (elevated temperatures) was recorded in UP2784 (69.66 %) while minimum in UP2748 (8.43 %) (**Figure 4.6.1**). Statistically values of MDA content at grain filling was significantly affected by sowing timings in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.6.1 Effect of differential temperatures on MDA content (μ M per g tissue) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

MDA content (μ M per g tissue)						
	At anthesis			15 DAA		
Varieties	**D1**		**D2**	**D1**		**D2**
UP2628	27.9		49.57	43.01		56.84
HD3086	31.96		46.50	41.86		57.05
UP2967	32.62		46.99	42.73		60.39
UP2784	30.00		41.95	40.28		68.32
UP2526	28.53		40.51	42.39		58.52
UP2565	20.21		27.00	41.40		44.95
UP2748	36.48		40.89	41.29		44.77
HD3059	40.34		45.00	42.24		49.25
	Treatment (T)	**Variety (V)**	**(TXV)**	**Treatment (T)**	**Variety (V)**	**(TXV)**
SEm ±	0.343	0.686	0.971	0.391	0.7821	1.106
CD (5%)	0.989	1.978	2.797	1.126	2.253	3.186

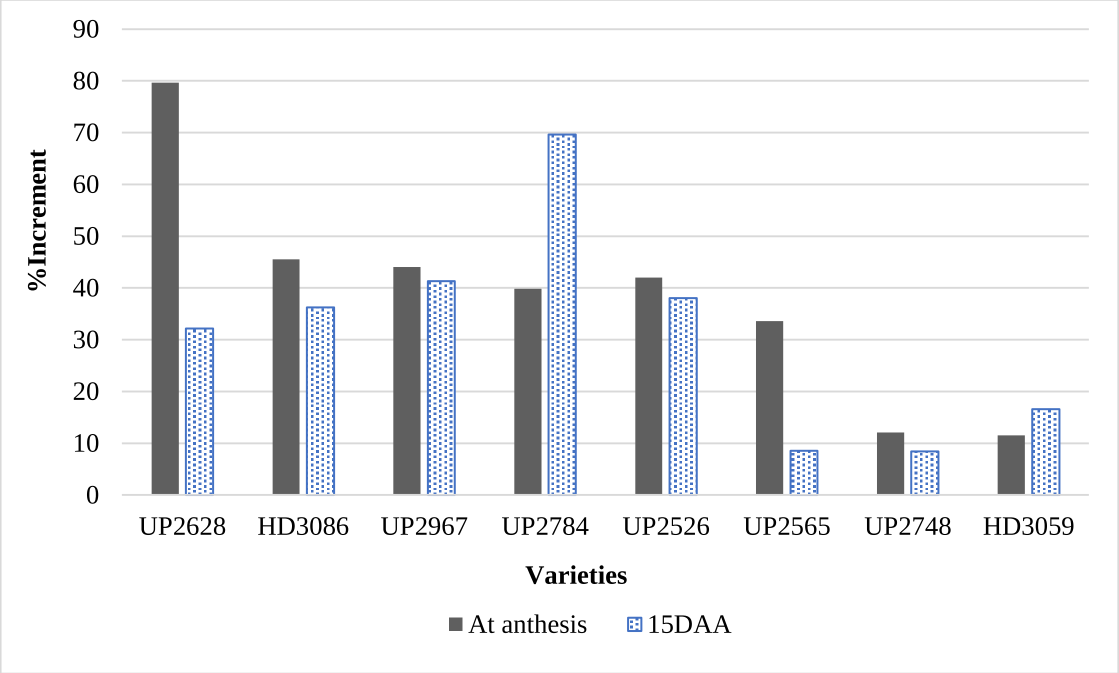


Figure 4.6.1 Percent increment in MDA content of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present study, MDA content in all wheat varieties was found higher under D2 and under grain filling stage (15 DAA) as compared to D1 and at anthesis stage respectively. From past reviews, Malondialdehyde assay, (MDA content) is considered as an indicator for lipid peroxidation in the cells which is caused by oxidative lipid injury underheat stress. Elevation in temperatures during sensitive growth stages of wheat life cycle which causes protein degradation, enzymes inactivation and loosening of cell membrane leads to increase in MDA content under stress condition (**Sairam *et al.*, 2004**). Similar results also followed by previous researches in which MDA content found higher due to elevation in air temperatures. According to one research, heat stress sowing conditions of wheat responsible for decrease in relative water content, increases in level of lipid peroxidation (Malondieldehyde) and H_2O_2 content in all the wheat cultivar namely C306, HD2285 and HD2329. This research also concludes that lower level of lipid peroxidation/ (MDA) content signifies higher degrees of heat tolerance and vice-versa (**Sairam *et al.*, 2004**). In another experiment which was done with different wheat varieties under two sowing conditions i.e. 27th November and 20th December. The results showed that MDA content was found increased by 4-fold under December sowing as compared to November sowing conditions. At anthesis, under November sown condition, highest MDA content observed in UP2338 (0.28 µ M) and lowest in UP2113 (0.20 µ M) while under December sowing the MDA content increased and found highest in UP2338 (0.35 µ M) and lowest in UP2113 (0.25 µ M). On comparing between the two sensitive stages, MDA content was found higher at the time of grain filling stage (20 days after anthesis) in comparison to anthesis stage under both sowing conditions. At grain filling stage (20 days after anthesis), under November sowing condition, highest MDA content observed in UP2338 (0.48 µ M) and lowest in UP2113 (0.38 µ M) while under December sowing MDA content increased and found highest in UP2338 (0.80 µM) and lowest in UP2113 (0.56 µ M). According to author, increased MDA content under elevated temperatures can be attributed to oxidation of linoleic and linolenic acids as the components of phosphor and galactolipids participating in the arrangement of reaction centres of photosystems in chloroplast membranes. As MDA is capable in interaction with free amino groups of proteins and with phospholipid molecules it can also initiate ethylene generation in membranes and put the cell under forced senescence resulting leakage of various ions through membranes **(Srivastava, 2005).** Similar study in which MDA content was also found increased by 4 folds under 19th December and 17th December sowing conditions in comparison to wheat cultivar sown on 25th November and 23rd November. Lower values of MDA content observed at the time of anthesis and after 10, 20 and 30 days after anthesis the MDA content was gradually found increased in all wheat varieties. Under November sowing condition (non-heat stress), highest MDA content was observed in UP2382 (0.29 unit/mg) and lowest observed in UP1109 (0.22 unit/mg) while December sowing conditions (elevated temperatures) MDA content was increased and highest observed in UP2382 (0.35 unit/mg) and lowest in UP1109 (0.26 unit/mg). Similarly, at the time of grain filling (20 days after anthesis) under November sowing, highest MDA content observed in VL802 (0.49 unit/mg) and lowest in UP1109 (0.39 unit/mg) while under December sowing conditions, highest value observed in UP2382 (0.81 unit/mg) and lowest in UP1109 (0.57 unit/mg). The reason for such increment in MDA content under elevation in temperatures according to the author is that due to under December sowing, plant experiences heat stress (elevation in temperature) during anthesis and after anthesis resulting (OH) radicles generated and attacks the unsaturated fatty acids chain of membrane lipids which can cause membrane leakage and increased MDA content (**Chandra, 2006)**. In one research, 36 different wheat genotypes were analysed under two sowing condition 20thNovember and 23rdDecember was done in order to check the effect of sowing condition on MDA content in leaves of wheat. The results showed that there was significant difference in MDA content of different wheat genotypes under December sowing as compared to November sowing and significant difference was also observed between the two sensitive stages of wheat life cycle (anthesis and grain filling). At the time of anthesis minimum MDA content observed in Raj3765, Raj4101, Lok54 and maximum MDA content observed in wheat genotypes HW5021 and HI1544. Genotypes Raj3765, Raj4101, Lok54 were considered as heat tolerant varieties with lower MDA content as due to heat stress there was less damage found in membranes of their leaves. According to the author the production of active oxygen species (AOS) creates oxidative stress to DNA proteins and lipids structure in plants which are exposed to high temperatures /any other stress full conditions. As MDA content is a prominent indicator of membrane impairments in plants showed higher values for more damage to the plants (**Dhyani, 2010).** An experiment conducted with winter wheat (*Triticum aestivium* L. cv. Yangmai 16). MDA concentration in wheat leaves significantly increases after a first treatment of day/night temperature of 32 ºC/28 ºC as compared to the control (day/night temperature of 24 ºC/20 ºC). After 10 days of anthesis, both control and first treatment samples were again treated with a post anthesis higher temperature stress in which day/night temperature of 34 ºC/30º C. The results showed that samples with only higher temperature stress after post anthesis showed significantly higher MDA concentration than samples that having pre-anthesis first heat treatment along with post anthesis heat stress. According to the author, it may be due to pre-anthesis heat stress of first treatment alleviated the negative effect of post anthesis severe heat stress (**Xin *et al*., 2016**). During an investigation, two cultivars of *Brassica Campestris* L.,"wucai" (heat sensitive and heat tolerant) were evaluated under elevated temperatures. Sensitive cultivar showed severe damage to photosynthetic apparatus and membrane system under heat stress as more accumulation of ROS and MDA were observed in sensitive cultivar as compared to tolerant (**Zou *et al*., 2017).** Lipid peroxidation was estimated during anthesis stage and grain filling stage in four different wheat varieties namely PBW343 & HD2329 (thermo-susceptible) and HD2985 & Halna (thermo-tolerant) under two treatments of 30 ºC (T1) for 2h and 38 ºC for 2h (T2) with a control of 22 ºC. The results showed that as the temperature increases MDA content/lipid peroxidation also increases in all four varieties. During grain filling, MDA content maximum in PBW343 and minimum in Halna (thermo-tolerant). Heat stress during grain filling causes denaturation of enzymes associated with source and sink. It also abrupt production of ROS which have damaging effect on key cellular organelles and membranes (**Kumar *et al*., 2018**). Two winter wheat varieties, Kraljica (normal sown variety) and Olimpija (late sown variety) were evaluated for their performance 0, 7, 14, 21, 28 and 35 days post-anthesis under similar sowing condition. The results showed that the Kraljica significantly increased MDA at the last sampling point, compared to Olimpija which shows that during grain filling (when temperature keeps on rising per day) the MDA content significantly increases in Olimpija as compared to Kraljica which can be used as an indicator of rapid senescence in Olimpija (**Spanic *et al.*, 2020).** A study in which four wheat varieties having difference in heat stress tolerance i.e. a high yielding, and heat tolerant: BARI Gom 26 (BG26), moderately heat tolerant: BARI Gom 25 (BG25) & BARI Gom 23 (BG23) and highly heat susceptible variety: Pavon 76 were evaluated for physiological and biochemical responses when they were exposed to 25º C (control) and 35 ºC (heat stress) condition. The results conclude that there was a percent increase in MDA content in "BG23" (42 %), "BG25" (39 %), "BG26" (38 %) and "Pavon" (108 %) compared to control. A greater increase (108 %) of MDA content was recorded in variety "Pavon" compared to control, indicated higher leakiness, lower heat-stability and higher fluidity of membrane compared to other varieties. On average, the other three varieties recorded 39.5% increase in MDA (**Mohi-Ud-Din *et al.*, 2021)**.

4.6.2 Superoxide dis mutase activity (SOD)

Superoxide dis mutase (SOD) activity of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling). SOD activity was found to be increased under D2 (elevated temperatures) as compared to D1. On comparing between the two stages, SOD activity in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage. In detailed view, at anthesis highest SOD activity was observed in UP2565 variety (0.45 Units) and lowest in UP2526 (0.12 Units) under D1 while under D2, highest value observed in UP2748 (0.54 Units) and lowest in UP2784 variety (0.22 Units) (**Table 4.6.2).** Maximum increment in SOD activity due to D2 (elevation in temperatures) was recorded in UP2526 (150 %) while minimum in HD3086 (7.32 %) (**Figure 4.6.2**). Statistically values of SOD activity at the time of anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest SOD activity was recorded in UP2565 variety (0.60 Units) and lowest in UP2628 (0.27 Units) under D1 while under D2, highest value of SOD was observed in UP2748 (0.64 Units) and lowest in UP2784 variety (0.37 Units)

(**Table 4.6.2).** Maximum increment in SOD due to D2 (elevation in temperatures) was recorded in UP2526 (72.73 %) while minimum in HD3086 (2.13 %) (**Figure 4.6.2**). Statistically values of SOD activity at the time of grain filling was significantly affected by differential sowing timings in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.6.2 Effect of differential temperatures on SOD activity (Units / gram sample) of different wheat varieties at anthesis and 15 DAA. *(D1 & D2 indicates sowing in the month of November and December respectively).*

SOD activity (Units / gram sample)				
	At anthesis		15 DAA	
Varieties	D1	D2	D1	D2
UP2628	0.18	0.23	0.27	0.41
HD3086	0.41	0.44	0.47	0.48
UP2967	0.28	0.35	0.49	0.61
UP2784	0.20	0.22	0.35	0.37
UP2526	0.12	0.30	0.33	0.57
UP2565	0.45	0.50	0.60	0.63
UP2748	0.43	0.54	0.58	0.64
HD3059	0.23	0.41	0.45	0.55

	Treatment (T)	Variety (V)	(TXV)	Treatment (T)	Variety (V)	(TXV)
SEm ±	0.0807	0.0161	.02285	0.0931	0.0186	0.02633
CD (5%)	0.0232	0.0465	0.0658	0.0268	0.0536	0.0758

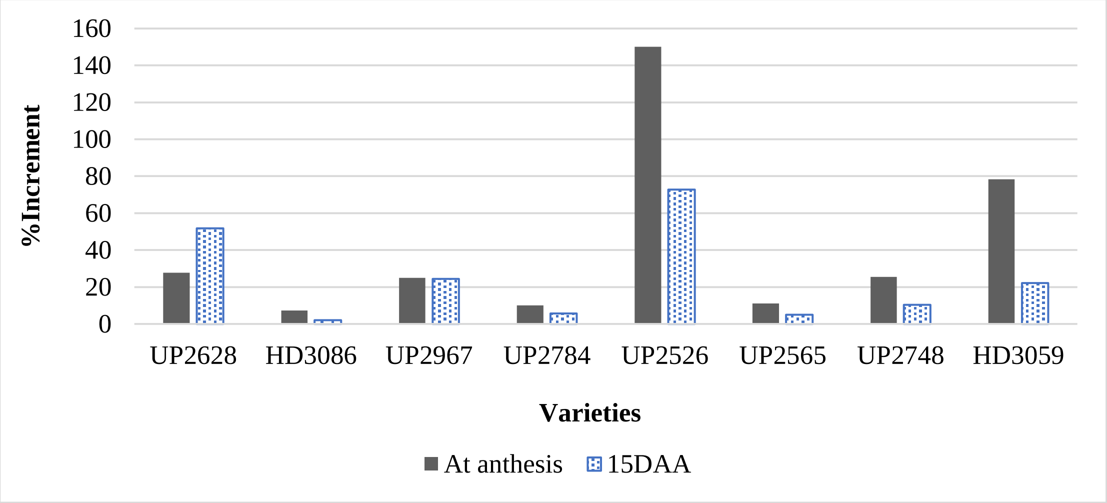


Figure 4.6.2 Percent increment in SOD activity of different wheat varieties under D2 in comparison to D1 condition. *(D1 & D2 indicates sowing in the month of November and December respectively).*

4.6.2.1 Specific enzyme activity for Superoxide dis mutase activity (SOD)

The specific enzyme activity for SOD was also calculated during both sowing conditions, it was observed that under D2, the specific enzyme activity was increased under D2 as compared to D1. On comparing between two stages, the specific enzyme activity for SOD was found higher during grain filling stage as compared to anthesis stage. At anthesis, under D1 maximum specific enzyme activity was observed for UP2565 (0.078 µ M /mg/min) and minimum for UP2526 (0.023 µ M /mg/min) while under D2, maximum activity observed for UP2565 (0.123 µ M /mg/min) and minimum for UP2628 (0.045 µ M /mg/min) **(Table 4.6.2.1**) Maximum increment in specific enzyme activity under D2 (elevated temperatures) as compared to D1 was observed for UP2526 (197.77 %) while minimum increment was observed for UP2784 (32.44 %) **(Figure 4.6.2.1).** Statistically specific enzyme activity for SOD at the time of anthesis was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). Similarly, at the time of grain filling, under D1 maximum specific enzyme activity was observed for UP2967 (0.105 µ M /mg/min) and minimum for UP2628 (0.052 µ M /mg/min) while under D2, maximum specific enzyme activity observed for UP2565 (0.155 µ M /mg/min) and minimum for UP2784 (0.076 µ M /mg/min) **(Table 4.6.2.1**). Maximum increment in specific enzyme activity at grain filling, under D2 as compared to D1 was observed for UP2526 (105.73 %) while minimum increment was observed for HD3086 (35.94 %) **(Figure 4.6.2.1)**. Statistically specific enzyme activity for SOD at the time of grain filling was significantly affected by differential sowing timings in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.6.2.1 Effect of differential temperatures on specific enzyme activity for SOD (µ M /mg/min) of different wheat varieties at anthesis and 15 DAA. *(D1 & D2 indicates sowing in the month of November and December respectively).*

SOD specific enzyme activity (µ M /mg/min)				
	At anthesis		**15 DAA**	
Varieties	**D1**	**D2**	**D1**	**D2**
UP2628	0.035	0.057	0.052	0.102
HD3086	0.072	0.102	0.082	0.112
UP2967	0.060	0.087	0.105	0.151
UP2784	0.034	0.045	0.059	0.076
UP2526	0.023	0.069	0.064	0.131
UP2565	0.078	0.123	0.104	0.155
UP2748	0.066	0.121	0.090	0.144
HD3059	0.036	0.089	0.071	0.120

	Treatment (T)	**Variety (V)**	**(TXV)**	**Treatment (T)**	**Variety (V)**	**(TXV)**
SEm ±	0.090	0.026	.0239	0.010	0.019	0.034
CD (5%)	0.034	0.055	0.078	0.038	0.067	0.088

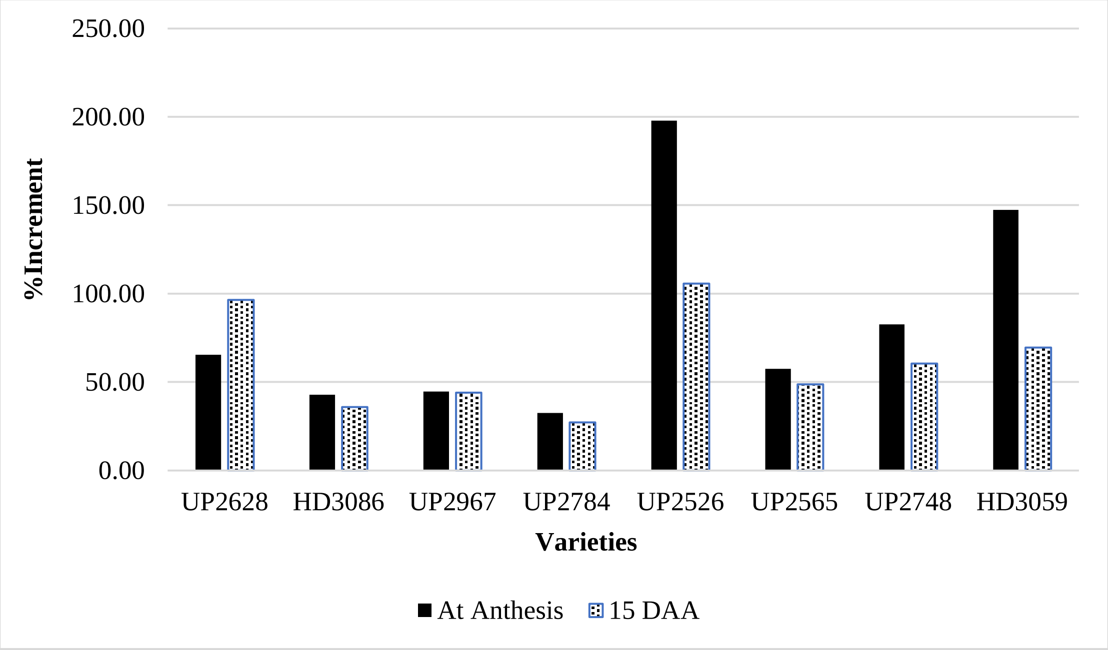


Figure 4.6.2.1 Percent increment in specific enzyme activity (SOD) of different wheat varieties under D2 in comparison to D1 condition. *(D1 & D2 indicates sowing in the month of November and December respectively).*

In present study, SOD activity in all wheat varieties were found significantly higher due to D2 and as well as under grain filling stage (15DAA) as compared to D1 and at anthesis stage respectively. Similar results also followed by previous researches in which SOD activity was found higher under elevated temperatures. In an experiment which was done with different wheat varieties under two sowing conditions i.e. 27th November and 20th December. The results showed that SOD activity was found upregulated under December sowing conditions as compared to November sowing conditions. SOD activity was also found upregulated by 20-25 % at grain filling stage as compared to anthesis stage under both sowing conditions. The reason for such increment according to the author is that higher the duration after anthesis it gets positively corelated with light temperature and humidity, stress and dry air. All such stresses switch on biosynthesis of enzymes which help in mitigating the adverse environmental variables to the greater extend. As heating causes an acceleration of the initiation reactions in the plant system and addition of antioxidants could takes place in the system (**Pokorný, 1986; Srivastava, 2005**). The results concludes that at anthesis, under November sown condition, highest SOD content observed in UP2338 (28.60 Units) and lowest in UP2113 (18.60 units) while under December sown, SOD content increased and found highest in UP2338 (30.50 units) and lowest in UP2113 (20.22 units). However, at grain filling stage (20 days after anthesis), under November sown condition, highest SOD activity observed in UP2338 (35.00 units) and lowest in UP2113 (25.60 units) while under December sown, SOD activity increased and found highest in UP2338 (45.00 units) and lowest in UP2113 (42.26 units) **(Srivastava, 2005).** Similar study in which SOD activity was also found increased by 3-4 folds under 19th December and 17th December in comparison to wheat cultivar sown on 25th November and 23rd November during two consecutive years. Lower values of SOD activity observed at the time of anthesis and after 10, 20 and 30 days after anthesis the SOD activity was gradually found increased in all wheat varieties. Under November sowing condition, highest SOD activity was observed in UP2382 (28.40 unit/mg) and lowest observed in UP1109 (20.04 unit/mg) while under December sowing, SOD activity was increased and highest observed in

UP2382 (31.46 unit/mg) and lowest in VL802 (26.05 unit/mg). Similarly, at the time of grain filling (20 days after anthesis) under November sowing, highest SOD activity observed in VL802 (43.84 unit/mg) and lowest in Raj 3675 (25.60 unit/mg) while under December sowing, highest value observed in UP2382 (48.02 unit/mg) and lowest in Raj 3675 (42.36 unit/mg). The reason for such increment in SOD activity under elevation in temperatures according to the author is that due to December sown plant experiences heat stress (elevation in temperature) during anthesis and after anthesis resulting antioxidant defence system of plants gets activated (in response to production of ROS) to minimize the oxidative damage. SOD is also an antioxidant and catalyses dismutation of superoxide radicles into ordinary oxygen molecule and hydrogen peroxide (**Chandra, 2006)**. According to some other reports, SOD activity was also found higher in tolerant wheat cultivar in comparison to susceptible cultivars and found increased in case of late and very late plantings conditions. Highest SOD activity was recorded at 15 days after anthesis in very late planting followed by anthesis stage and lowest activity was recorded at vegetative stage (**Almenselmania *et al*., 2009**). A study in another crop i.e sunflower plants (*Helianthus annuus* L.) which is treated under elevated temperatures stress by exposing plants to 35 and 45 °C for 12 hours and under controlled condition (27 ºC). The results concludes that high-temperature stress significantly increased leaf SOD activity as compared with the control. It was also noted that plants under controlled conditions had the minimum value for SOD enzyme activity while the maximum values observed for heat treated plants. High SOD activities, indicating that there was efficient ROS scavenging activity in the system. The action of SOD helps in efficiently eliminates the superoxide ions and indirectly protects plants against more toxic hydroxyl radicals. Excessive ROS production can cause oxidative stress, which damages plants; therefore, ROS-scavenging antioxidant enzymes, such as SOD plays a vital role in removing these destructive oxidant species. By catalysing the detoxification of O^{2-} to O^2 and H_2O_2, SOD blocks cell damage caused by O^{2-}. Antioxidant defence systems play vital roles in helping plants tolerate stressful conditions **(Akladious, 2014**). An experiment was conducted in two wheat cultivars HD2985 (thermo-tolerant) and

HD2329 (thermo-susceptible) under controlled environment (22ºC) and heat treatment with an increase of 1ºC/10 minutes till the temperature reaches 38ºC for 2 hours at the time of anthesis. The results indicate that the Cu/Zn SOD activity showed 4.1 folds upregulation in heat treated HD2329 (thermo-susceptible) and 1.2 folds down regulation in HD2985 (thermo-tolerant) wheat cultivars. This is may be due to the diversity in the heat tolerant capacity of different wheat germplasm. Along with that the maximum SOD activity in HD2985 was 14.1 Units and in HD2329 was 9.5 units in response to treatment of 38ºC for 2 hours. This concludes that the activity of defence/ stress associated enzymes (SOD) increases with the elevation in temperature and it is greater in case of tolerant cultivars and lesser in case of susceptible cultivar **(Kumar *et al*., 2018).** An experiment was done to evaluate the effect of terminal heat stress on superoxide dis mutase (SOD) activity in wheat variety (Faisalabad-2008) with treatments; control, terminal drought stress alone (50% field capacity during reproductive phase), terminal heat stress alone (wheat grown inside plastic tunnel during reproductive phase) and terminal drought stress + terminal heat stress. The SOD activity was found significantly increased with exposure of stresses and the highest SOD activity (54%) was observed under combined stress condition. As ROS generation due to heat stress induces the production of abscisic acid that act as a signal molecule under stressed conditions and regulate the gene expressions that control the production of enzymatic antioxidants such as superoxide dismutase (**Sattar *et al*., 2020**). A new potential biochemical marker (Mn-SOD) gene of ~733 nucleotide long is identified from wheat *cv*. HD2985. The location of the gene is on Chromosome 6D. Maximum expression and activity of Mn-SOD was observed in leaves of wheat *cv.* Raj3765 in late grain filling stage as compared to stem and spike under heat stress condition that shows Mn-SOD is directly involved in thermotolerance in wheat (**Kumar *et al*., 2020**). A study in which four wheat varieties having difference in heat stress tolerance i.e. a high yielding, and heat tolerant: BARI Gom 26 (BG26), moderately heat tolerant: BARI Gom 25 (BG25) & BARI Gom 23 (BG23) and highly heat susceptible variety: Pavon 76 were evaluated for physiological and biochemical responses when they were exposed to 25º C (control) and 35 ºC (heat stress) condition. The results conclude that there was a significant percent increase in SOD activity in "BG23" (16 %), "BG25" (19 %), "BG26" (36%) but not in "Pavon" (8%) (**Mohi-Ud-Din *et al*., 2021)**.

4.6.3 Catalase activity (CAT)

Catalase (CAT) activity of each wheat varieties was observed at the time of anthesis and 15 days after anthesis (grain filling). CAT activity was found to be increased under D2 (elevated temperatures) as compared to D1 conditions. On comparing between the two stages, CAT activity in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage. In detailed view, at anthesis highest CAT activity was observed in UP2748 variety (0.97µ M) and lowest in UP2565 (0.25 µ M) under D1 while under D2, highest value observed in UP2748 (1.24 µ M) and lowest in UP2628 variety (0.38 µ M) (**Table 4.6.3).** Maximum increment in CAT activity due to D2 (elevated temperatures) was recorded in UP2565 (340 %) while minimum in UP2967 (2.86 %) (**Figure 4.6.3**). Statistically values of CAT activity at anthesis was significantly affected by D2 in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). At grain filling (15 DAA) highest CAT activity was recorded in UP2565 variety (1.64 µ M) and lowest in UP2628 (0.53 M) under D1 while under D2, highest value of CAT was observed in UP2748 (2.63 µ M) and lowest in UP2784 variety (0.69 µ M) (**Table 4.6.3).** Maximum increment in CAT due to D2 (elevated temperature) was recorded in UP2748 (108.33 %) while minimum in UP2784 (1.31 %) (**Figure 4.6.3**). Statistically, values of CAT activity at the time of grain filling were significantly affected by D2 in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.6.3 Effect of differential temperatures on CAT activity (µ M OD H2O2 DISMUTED) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="9">CAT activity (µ M OD H2O2 DISMUTED</th></tr>
<tr><th colspan="5">At anthesis</th><th colspan="4">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th colspan="2">D2</th><th colspan="2">D1</th><th colspan="2">D2</th></tr>
<tr><td>UP2628</td><td colspan="2">0.29</td><td colspan="2">0.38</td><td colspan="2">0.53</td><td colspan="2">0.95</td></tr>
<tr><td>HD3086</td><td colspan="2">0.63</td><td colspan="2">0.65</td><td colspan="2">0.85</td><td colspan="2">1.10</td></tr>
<tr><td>UP2967</td><td colspan="2">0.70</td><td colspan="2">0.72</td><td colspan="2">0.96</td><td colspan="2">1.38</td></tr>
<tr><td>UP2784</td><td colspan="2">0.30</td><td colspan="2">0.44</td><td colspan="2">0.68</td><td colspan="2">0.69</td></tr>
<tr><td>UP2526</td><td colspan="2">0.53</td><td colspan="2">1.09</td><td colspan="2">1.09</td><td colspan="2">1.26</td></tr>
<tr><td>UP2565</td><td colspan="2">0.25</td><td colspan="2">1.10</td><td colspan="2">1.64</td><td colspan="2">2.26</td></tr>
<tr><td>UP2748</td><td colspan="2">0.97</td><td colspan="2">1.24</td><td colspan="2">1.26</td><td colspan="2">2.63</td></tr>
<tr><td>HD3059</td><td colspan="2">0.46</td><td colspan="2">0.76</td><td colspan="2">0.88</td><td colspan="2">1.72</td></tr>
<tr><td></td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.0105</td><td colspan="2">0.0211</td><td>0.0299</td><td>0.413</td><td colspan="2">0.082</td><td>0.116</td></tr>
<tr><td>CD (5%)</td><td>0.0304</td><td colspan="2">0.0609</td><td>0.0862</td><td>0.119</td><td colspan="2">0.238</td><td>0.336</td></tr>
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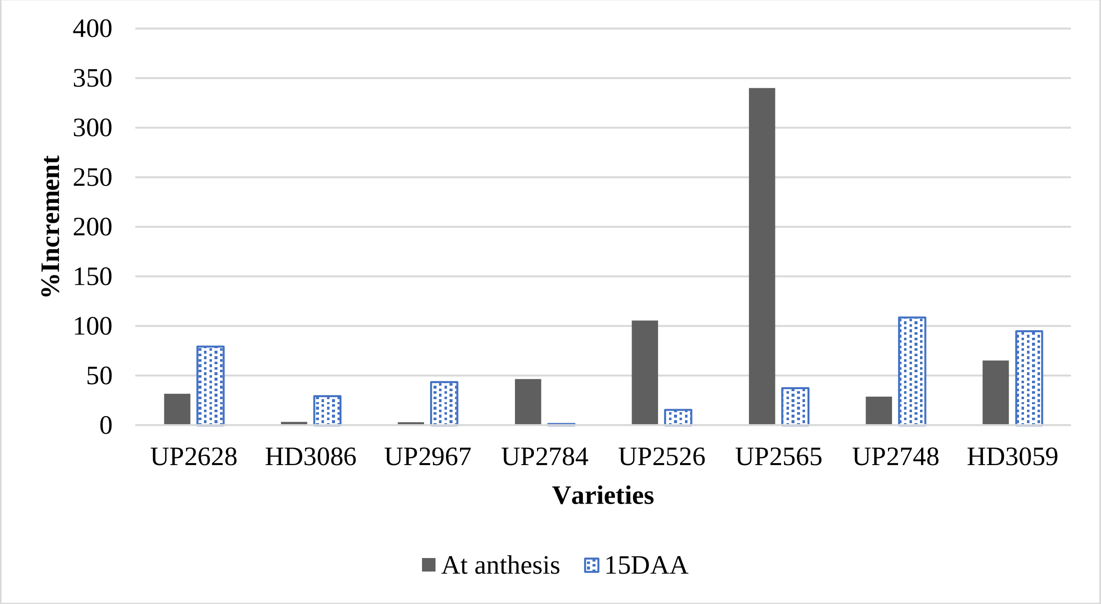

Figure 4.6.3 Percent increment in CAT activity of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

4.6.3.1 Specific enzyme activity for Catalase (CAT)

The specific enzyme activity for CAT was also calculated during both sowing conditions, it was observed that under D2, the specific enzyme activity was increased under D2 as compared to D1. On comparing between two stages, the specific enzyme activity for CAT was found higher during grain filling stage as compared to anthesis stage. At anthesis, under D1 maximum specific enzyme activity was observed for UP2748 (0.125 µ M /mg/min) and minimum for UP2628 (0.030 µ M /mg/min) while under D2, maximum activity observed for UP2748 (0.139 µ M /mg/min) and minimum for UP2628 (0.031 µ M /mg/min) (**Table 4.6.3.1**). Maximum increment in specific enzyme activity under D2 (elevated temperatures) as compared to D1 was observed for UP2565 (210.61 %) while minimum increment was observed for UP2628 (1.66 %) (**Figure 4.6.3.1**). Statistically specific enzyme activity for CAT at anthesis was significantly affected by D2 in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$). Similarly, at the time of grain filling, under D1 maximum specific enzyme activity was observed for UP2565 (0.179 µ M /mg/min) and minimum for UP2628 (0.077 µ M /mg/min) while under D2, maximum specific enzyme activity observed for UP2748 (0.235 µ M /mg/min) and minimum for UP2628 (0.077 µ M /mg/min) (**Table 4.6.3.1)**. Maximum increment in specific enzyme activity at grain filling, under D2 as compared to D1 was observed for UP2748 (43.59 %) while minimum increment was observed for UP2565 (2.36 %) (**Figure 4.6.3.1)**. Statistically, specific enzyme activity for CAT at the time of grain filling were significantly affected by D2 in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatments × varieties were also found significant at ($p \leq 0.01$).

Table 4.6.3.1 Effect of differential temperatures on specific enzyme activity for CAT (µ M /mg/min) of different wheat varieties at anthesis and 15 DAA. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="7">CAT specific enzyme activity (µ M /mg/min)</th></tr>
<tr><th colspan="4">At anthesis</th><th colspan="3">15 DAA</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th colspan="1">D2</th><th colspan="2">D1</th><th>D2</th></tr>
<tr><td>UP2628</td><td colspan="2">0.030</td><td>0.031</td><td colspan="2">0.055</td><td>0.077</td></tr>
<tr><td>HD3086</td><td colspan="2">0.072</td><td>0.086</td><td colspan="2">0.090</td><td>0.095</td></tr>
<tr><td>UP2967</td><td colspan="2">0.065</td><td>0.070</td><td colspan="2">0.090</td><td>0.111</td></tr>
<tr><td>UP2784</td><td colspan="2">0.035</td><td>0.043</td><td colspan="2">0.060</td><td>0.080</td></tr>
<tr><td>UP2526</td><td colspan="2">0.055</td><td>0.095</td><td colspan="2">0.113</td><td>0.118</td></tr>
<tr><td>UP2565</td><td colspan="2">0.029</td><td>0.089</td><td colspan="2">0.179</td><td>0.183</td></tr>
<tr><td>UP2748</td><td colspan="2">0.125</td><td>0.139</td><td colspan="2">0.163</td><td>0.235</td></tr>
<tr><td>HD3059</td><td colspan="2">0.059</td><td>0.070</td><td colspan="2">0.112</td><td>0.158</td></tr>
<tr><td></td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.0205</td><td>0.0310</td><td>0.0398</td><td>0.051</td><td>0.092</td><td>0.201</td></tr>
<tr><td>CD (5%)</td><td>0.0404</td><td>0.0706</td><td>0.0952</td><td>0.219</td><td>0.308</td><td>0.486</td></tr>
</table>

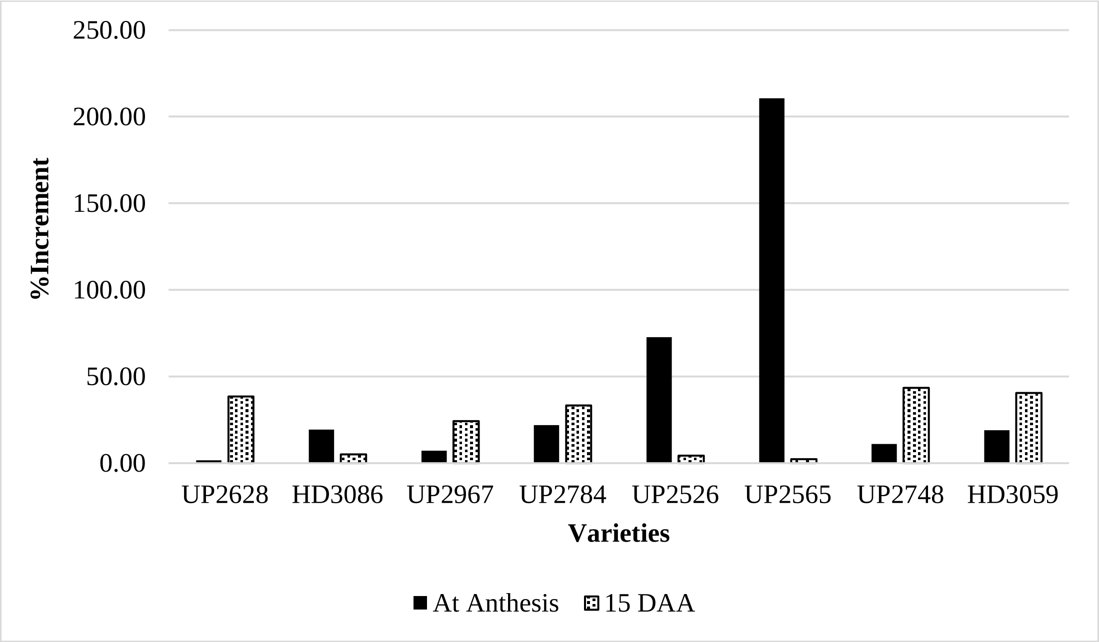

Figure 4.6.3.1 Percent increment in specific enzyme activity (CAT) of different wheat varieties under D2 in comparison to D1. *(D1 & D2 indicates sowing in the month of November and December respectively).*

In present study, CAT activity in all wheat varieties were found significantly higher due to D2 condition and as well as under grain filling stage (15 DAA) as compared to D1 and at anthesis stage respectively. Similar results also followed by previous researches in which CAT activity found higher under elevation in air temperatures. CAT activity catalyses the decomposition of hydrogen peroxide to oxygen. According to one study heat treatment increased the CAT activity in T. *aestivum* genotype whereas, decreased in T. *durum* genotype of wheat (**Keles and Oncel., 2002**). An experiment conducted with wheat cultivar (C306) (thermotolerant) to study the effect of differential heat shocks on its enzyme activity (Catalase activity). In this study, wheat variety was grown in net house as well as in growth chamber inside a phytotron under controlled conditions with optimum temperature of 26/22 °C and under heat stress conditions of different temperatures (22, 30, 35 and 40 ºC). The results conclude that there was an increase in CAT activity observed in samples collected from net house at different stages of growth and development. At vegetative stage, CAT activity was 4.62 Units which increased to 9.4 Units during anthesis and 11.34 Units during grain filling- milky dough stage. Further, a decrease in CAT activity was observed during the seed maturation and was found 9.31 Units. The activity of CAT was 21.35 Units in 22 °C, 23.57 Units in 30 °C, 31.53 Units in 35 °C and 40.92 Units in 40 °C was also observed. The highest activity of CAT was observed in response to heat shock treatment of 40 °C for 2 h. Increase in CAT activity could play a role in the protection of the plants from the damages of upward accumulation of H_2O_2 in wheat leaves in response to heat shock. From this study, it is suggested that there is a potential role for antioxidant enzymes such as CAT in the reduction of elevated levels of H_2O_2 in wheat plants grown under heat stress condition **(Kumar *et al.*, 2012).** A study in another crop sunflower plants (*Helianthus annuus* L) which is treated under elevated temperatures stress by exposing plants to 35 and 45 °C for 12 hours. High-temperature stress significantly increased leaf SOD and CAT activity as compared with the control. Control plants had the minimum value for SOD and CAT enzyme while the maximum values observed for heat treated samples. High SOD and CAT activities, indicating that there was efficient ROS scavenging activity in the system. The combined action of SOD and CAT efficiently eliminates hydrogen peroxide and superoxide and indirectly protects plants against more toxic hydroxyl radicals. Excessive ROS production can cause oxidative stress, which damages plants; therefore, ROS-scavenging antioxidant enzymes, such as SOD, CAT, play a vital role in removing these destructive oxidant species. By catalysing the detoxification of $O_2{\cdot}^-$ to O_2 and H_2O_2, SOD blocks cell damage caused by O2·−. CAT break down H_2O_2 to H_2O and O_2. When plants are exposed to stress, antioxidant systems become active and begin to scavenge ROS. Antioxidant defence systems play vital roles in helping plants tolerate stressful conditions **(Akladious, 2014**). During an experiment it was investigated that there was high antioxidant potential in thermo-tolerant wheat genotype as compared to thermo-susceptible. During pollination a significant increase in CAT activity was observed in Halna (thermo-tolerant) in response to 30 ºC; T1treatment (15.8 µg/ mg protein) and 38ºC; T2 treatment (16.4 µg/ mg protein) as compared to other cultivar, CAT activity was found very low in HD 2329 (thermo-susceptible under T1 (8.12 µg/ mg protein) and T2 treatment (8.65 µg/ mg protein). During grain filling, maximum cat activity observed in Halna (18.4 µg/ mg protein) and minimum in PBW343 (9.2µg/ mg protein) in response to T2 (**Kumar *et al*., 2018**). An experiment was conducted in two wheat cultivars HD2985 (thermo-tolerant) and HD2329 (thermo-susceptible) under controlled environment (22 ºC) and heat treatment with an increase of 1 ºC/10 minutes till the temperature reaches 38 ºC for 2 hours at the time of anthesis. The results indicate that the that the maximum CAT activity in HD2985 was 6.2 Units and in HD2329 was 5.6 units in response to treatment of 38ºC for 2 hours. This concludes that the activity of defence/stress associated enzymes (CAT) increases with the elevation in temperature and it is greater in case of tolerant cultivars and lesser in case of susceptible cultivar **(Kumar *et al*., 2018).** A wheat variety was subjected to various combinations of heat treatment in comparison to control led to conclude that under elevated temperature catalase activity in plant was increased (61%) to compensate the bad effect of ROS and convert harmful active species to inactive condition. (**Sattar *et al*., 2020**). An experiment was done to evaluate the biochemical responses in contrasting thermo-tolerant BARI Gom 26 (“BG26”) and heat susceptible Pavon 76 (“Pavon”) wheat varieties when they were exposed to 25°C (control) and 35°C (heat stress), stress condition during the seedling stage. The results showed that the tolerant variety maintained its thermo-stability under heat stress condition by accumulation more antioxidant activity or least reduction in CAT activity (15 to 20%) as compared to the heat- susceptible where the reduction in CAT activity due to heat stress was found 38% in comparison to control (**Mohi-ud-Din *et al*., 2021**).

Correlation analysis between biochemical parameters and antioxidants with grain yield under both sowing condition

Correlation was also analysed for biochemical parameters and antioxidants with grain yield under both sowing conditions. Under D1, grain yield showed negatively but significant correlation with proline content (r=-0.88**) and Catalase enzyme activity (r=-0.75*) while positive but non-significant correlation of grain yield was observed with total chlorophyll and NR activity. Under D2, negative but significant correlation of grain yield with total chlorophyll (r=-0.78*) and carotenoids (r=-0.78*) was observed, showing reduction in chlorophyll content and carotenoids due to heat stress which causes reduced photosynthesis and ultimately grain yield. A positive correlation of grain yield with NR activity was also observed under D2 which could be responsible for least reduction in grain yield under elevated temperatures by some of the wheat varieties. On comparing between the other biochemical and antioxidant parameters, it can be concluded that under D1, negative but significant correlation was observed between total chlorophyll and Superoxide dis mutase (SOD) activity (r=-0.71*). While positive and significant correlation was observed between carotenoid and MDA content (r=0.74*) and proline &CAT activity (r=0.77*). Under D2, positive but significant correlation was observed between carotenoids and SOD activity (r=0.77*), between proline and catalase (r=0.80*) and between SOD and CAT (r=0.74*). From this relationship it can be concluded that when plant experiences heat stress, the antioxidant defence system and osmolytes in plants get activated and enhanced under D2 as compared to D1 (**Table 4.6.4.1 and 4.6.4.2).**

Table 4.6.4.1 Pearson’s correlation between biochemical parameters and antioxidants with grain yield under D1.

	GY	*TC*	*CTN*	*PRO*	*NR*	*MDA*	*SOD*	*CAT*
GY	1							
TC	0.37	1						
CTN	-0.10	-0.28	1					
PRO	-0.88**	-0.46	0.37	1				
NR	0.03	-0.23	-0.11	-0.35	1			
MDA	-0.27	0.11	0.74*	0.47	-0.46	1		
SOD	-0.39	-0.71*	0.32	0.57	-0.08	-0.10	1	
CAT	-0.75*	-0.21	0.36	0.77*	-0.22	0.60	0.36	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Total Chlorophyll (TC), Carotenoid content (CTN), Proline content (PRO), Nitrate Reductase activity (NR), Malondialdehyde Assay (MDA), Superoxide-dis mutase (SOD) and Catalase activity (CAT).

Table 4.6.4.2 Pearson’s correlation between biochemical parameters and antioxidants with grain yield under D2.

	GY	*TC*	*CTN*	*PRO*	*NR*	*MDA*	*SOD*	*CAT*
GY	1							
TC	-0.78*	1						
CTN	-0.78*	0.64	1					
PRO	-0.40	0.68	0.41	1				
NR	0.61	-0.52	-0.57	0.05	1			
MDA	0.01	-0.35	-0.13	-0.58	-0.04	1		
SOD	-0.48	0.35	0.77*	0.46	-0.06	-0.48	1	
CAT	-0.45	0.56	0.44	0.80*	0.24	-0.63	0.74*	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Total Chlorophyll (TC), Carotenoid content (CTN), Proline content (PRO), Nitrate Reductase activity (NR), Malondialdehyde Assay (MDA), Superoxide-dis mutase (SOD) and Catalase activity (CAT).

4.7 Effect of elevated temperatures on seed quality of wheat.

4.7.1 Percentage of total soluble carbohydrates and starch content

Total soluble carbohydrates and starch content in seeds of each wheat varieties was observed under both sowing sowings conditions. Total soluble carbohydrates and starch content were found reduced under D2 (elevated temperatures) as compared to D1. Under D1, maximum carbohydrates content in seeds was observed in UP2967 variety (86.19 %) and minimum in UP2565 (72.34 %) while under D2, maximum carbohydrates content observed in UP2748 variety (73.94 %) and minimum in UP2628 (60.40 %) (**Table 4.7.1)**. Maximum percent decrease in total soluble carbohydrates was observed in UP2628 variety (24.36 %) and minimum in HD3059 (2.64 %) (**Figure 4.7.1)**. Statistically carbohydrates were significantly affected by elevation in temperatures in terms of treatments at ($p \leq 0.01$) while in terms of varieties the carbohydrates were found non-significantly affected by elevated temperatures ($p > 0.05$). Interaction between treatment × varieties were also found non-significant at ($p > 0.05$). For starch content, under D1, highest starch content in seeds was observed in UP2967 variety (77.57 %) and lowest in UP2565 (65.10 %) while under D2, highest starch content observed in UP2748 variety (66.54 %) and lowest in UP2628 (54.36 %) (**Table 4.7.1)**. Maximum percent decrease in starch content was observed in UP2628 variety (24.36 %) and minimum in HD3059 (2.64 %) (**Figure 4.7.1)**. Statistically starch content were significantly affected by elevated temperatures in terms of treatments at ($p \leq 0.01$) while in terms of varieties the starch content were found non-significantly affected by elevated temperatures ($p > 0.05$). Interaction between treatment × varieties were also found non-significant at ($p > 0.05$).

Table 4.7.1 Effect of differential temperatures on total carbohydrates (%) and starch content (%) in grains of different wheat varieties. *(D1& D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="4">Carbohydrates (%)</th><th colspan="3">Starch Content (%)</th></tr>
<tr><th>Varieties</th><th colspan="2">D1</th><th>D2</th><th colspan="2">D1</th><th>D2</th></tr>
<tr><td>UP2628</td><td colspan="2">79.87</td><td>60.40</td><td colspan="2">71.88</td><td>54.36</td></tr>
<tr><td>HD3086</td><td colspan="2">79.22</td><td>61.44</td><td colspan="2">71.30</td><td>55.30</td></tr>
<tr><td>UP2967</td><td colspan="2">86.19</td><td>69.13</td><td colspan="2">77.57</td><td>62.22</td></tr>
<tr><td>UP2784</td><td colspan="2">75.46</td><td>64.97</td><td colspan="2">67.91</td><td>58.47</td></tr>
<tr><td>UP2526</td><td colspan="2">74.58</td><td>70.25</td><td colspan="2">67.12</td><td>63.23</td></tr>
<tr><td>UP2565</td><td colspan="2">72.34</td><td>62.17</td><td colspan="2">65.10</td><td>55.95</td></tr>
<tr><td>UP2748</td><td colspan="2">79.22</td><td>73.94</td><td colspan="2">71.30</td><td>66.54</td></tr>
<tr><td>HD3059</td><td colspan="2">75.62</td><td>73.62</td><td colspan="2">68.06</td><td>66.26</td></tr>
<tr><td></td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td>Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>2.454</td><td>4.909</td><td>6.943</td><td>2.209</td><td>4.418</td><td>6.248</td></tr>
<tr><td>CD (5%)</td><td>7.071</td><td>14.142</td><td>20.000</td><td>6.364</td><td>12.728</td><td>18.000</td></tr>
</table>

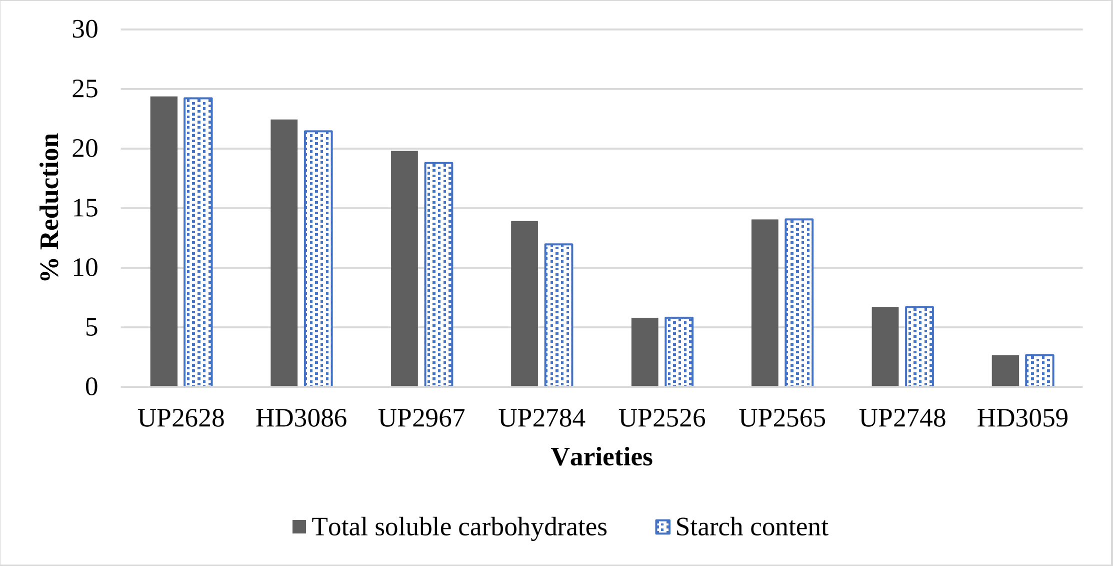

Figure 4.7.1 Percent reduction in total soluble carbohydrates and starch content in grains of different wheat varieties under D2 in comparison to D1. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present study, total soluble carbohydrates in seeds were found negatively affected by D2 (elevation in temperatures) in all wheat varieties as compared to D1. From previous studies carbohydrates is considered as a major component of assimilation flux in filling grains. Under heat stress, duration of grain filling was found to be reduced due to forced maturity/senescence led to also have a bad effect on carbohydrate metabolism in wheat kernel hence reduction in total soluble carbohydrates observed under elevated temperatures in wheat. According to a research, carbohydrate metabolism pathway such as sugar conversions, glycolysis, TCA cycle and lipid metabolism were found decreased under heat stress condition as compared to non-stressed (**Zahedi *et al.*, 2003**). In an experiment the effect of terminal heat stress on carbohydrate accumulation on three wheat cultivars (BARI Gom 25, BARI Gom 26 and Pavon 76) under sown on 18 November, 2011 under two temperature conditions normal (23°C in open field) and elevated (6 ± 1°C higher than open field mean air temperature by polythene chamber) were studied. The results showed that elevation in temperature shortened the grain filling duration by 5-day in BARI Gom 25 and BARI Gom 26 and by 10-day in Pavon 76. A higher level of grain soluble sugar was found up to 20 DAA (20 days after anthesis) in BARI Gom 26 under open field temperature condition compared to elevated temperature condition, which was found in Pavon 76 only for15-day. Premature of grain development under heat stress condition had been reported due to limited enzymatic function involved in sugar to starch conversion. It had appeared that elevated temperature restricted starch synthesis more prominent in heat sensitive cultivars compare to heat tolerant cultivars (**Khatun and Ahmed 2015**). In another experiment in which the effect of three planting dates; 25th October, 25th November and 25th December on carbohydrate content of four bread wheat (*Triticum aestivum* L.) cultivars; Shandaweel 1, Sids 12, Giza 168 and Sakha 93, during the two successive growing seasons of 2012/2013 and 2013/2014 were studied. The results showed that the delay in sowing negatively affected the percentage of total soluble carbohydrate during both consecutive years 2012 and 2013. In all wheat varieties, percentage of grain carbohydrates was found reduced as the sowing dates increases from October to November and November towards December, except in Sakha 93 in which % carbohydrates were increased under November (74.93 %) as compared to October sowing (70.93), however in December sowing again % of carbohydrates was found reduced (65.01 %). On an average of two years data, carbohydrate % under November sowing was found within the range of 73.82 % to 70.95 % while under October sowing conditions, the range was 74.93% to 71.69% and under December it was reduced to a range between 71.63 % to 65.01 %. The reduction in the carbohydrate percentage in the produced wheat grains after the exposure to high temperature stress may be attributed to a reduction in endosperm cell size under heat stress **(El Maghraby *et al.*, 2016**). Sucrose is the main photosynthetic product of leaves and key enzyme sucrose synthase (SS) necessary for sucrose to enter into various metabolic pathways, SS plays important role in plants growth and development. In an experiment, winter wheat cultivar Gaocheng 8901 was grown under two different sowing conditions one as a controlled (25ºC/18ºC day and night temperatures) and another as a treatment of (40ºC for 2hours from flowering to maturity). The results showed that the SS expression was found to down regulated under heat stress condition which indirectly inhibits the carbohydrate synthesis and thus reducing the wheat yield and quality. Proteomics studies also confirmed that Glucose 6 phosphate dehydrogenase which is a key regulatory enzyme in plant pentose phosphate pathway, an important pathway for carbohydrate metabolism was also found down regulated under heat stress condition as a result carbohydrate accumulation found reduced under heat stress condition (**Zhang *et al.*, 2018**). Similar results in which *Chinese spring* wheat kernel was evaluated to study the changes in metabolic proteins under heat treatment (37/17 ºC) and control condition (24ºC/17 ºC). The results concludes that due to heat stress there was an abundant decrease in heat responsive proteins which involved in carbohydrates metabolism due to which metabolic process of sucrose, starch and amino acid showed a significant decrease under heat stress condition. In the same research, metabolites related to sugar conversion, glycolysis, TCA cycle and lipid metabolism were studied and it was found that 16 out of 21 metabolites related to sugar conversion, glycolysis, TCA cycle and lipid metabolism were decreased under heat stress as compared to non-stress condition. These results indicate that less assimilation allocated to grains causes less carbohydrate content in grains (**Wang *et al.*, 2018**). Similarly, 237 spring wheat varieties under different sowing months (October) & (February) as normal and late sown condition were studied. The metabolomic analysis demonstrated the decrease in 16 out of 21 metabolites related to carbohydrate metabolism pathway under elevated temperature were observed reduced due to late sowing. As carbohydrates are well known major component of assimilation flux in filling grains, but due to elevated temperature led to less assimilation allocated to grains causes less carbohydrate content in grains However, G1P (Glucose 1 Phosphate) and G6P (Glucose 6 Phosphate), the carbohydrate precursor for starch synthesis remains stable in heat stress which is found responsible for stable filling rate during elevation in temperatures (**Wang *et al.*, 2018**). An experiment conducted in two wheat cultivars i.e HD2985 (thermotolerant) and HD2329 (thermo-susceptible) under two conditions i.e- controlled environment (22 ºC) and under heat stress condition (at 38 °C, 2h treatment at grain filling stage). The results concluded that under heat stress condition there was 15% decrement in carbohydrate metabolism process observed in comparison to controlled condition. From the functional cataloguing of stress associated active protein (SAAPs) concludes that the carbon assimilatory pathway is most altered under stress condition as compared to controlled which is followed by starch and sucrose metabolism. Pathways involving photosynthesis, glyoxylate and decarboxylate pathway, glycolysis and gluconeogenesis pathway found adversely affected and altered under stress condition. An increase in degradative enzymes (α/β amylase) and alteration in enzymes related to carbohydrate metabolism such as fructose 1-6 bis phosphate, 1-phosphatase, phosphoric ester hydrolase, chitinase, hydrolase, beta mannosidase, Rubisco and RubisCo activase observed under heat stress condition as compared to controlled condition. Thermo-susceptible cultivar HD2329 showed down regulation of SAAPs associated with carbohydrate metabolism and lipid metabolism under heat stress condition (**Kumar *et al*., 2019**).

In the present study, the accumulation of starch content in wheat kernel at maturity was found negatively affected and decreased under D2 as compared to D1. Starch accounts about 70 % of wheat grain dry weight and reduction in starch deposition is the main reason for reduced grain weight under heat stress condition (**Bhullar and Jenner, 1985**). From previous studies it is well known that at higher temperatures (above than optimum temperatures i.e 22ºC during grain filling of wheat) hydrogen bonds in starch became weakened and broken, resulting exposing of amylose and amylopectin and allowing amylase to hydrolyses the glycosidic bonds. Hence, starch content found to be reduced under elevated temperatures. From previous researches, there are some enzymes which involved in starch biosynthesis in developing wheat kernel found sensitive towards elevated temperatures such as sucrose synthase, soluble starch synthase and granule bound starch synthase found adversely affected due to the sudden rise in air temperatures (above 18 to 22 ºC) and causes reduction in starch biosynthesis and its deposition in wheat kernels especially under elevated temperature conditions of wheat (**Spiertz *et al*., 2006**). In another study on wheat showed that the rate of starch synthase is mainly determined by enzyme sucrose synthase. However, soluble starch synthase (SSS) also regulates starch synthesis and also found highly sensitive towards elevated temperatures. Under heat stress, the activity of Soluble starch synthase was found decreased which reduces starch accumulation in wheat kernel (**Prakash *et al*., 2003**). During an experiment, 32 wild and cultivated genotypes of wheat species belonging to diploid (donors may be of B, A and D genomes), tetraploid (BBAA and AAGG genomes) and hexaploid

(BBAADD genome) were evaluated for heat stress tolerance under two dates i.e 18 November (normal sowing) and 15 January (heat stress). The results concludes that under heat stress the grain starch content was found reduced by 28.9 % in diploid, 22.30 % in tetraploid and 19.50 % in hexaploidy wheat genotypes as compared to normal sowing. On comparing within the three wheat genotype groups, hexaploid and tetraploid group of wheat species showed 2.6-fold higher starch content than diploid wheat species. However, hexaploids had the highest grain starch content followed by tetraploid and diploid (**Chinnusamy and Chopra, 2003**). The influence of high temperature on quality of starchwas studies through an experiment in which two bread wheat varieties (Kariega and SST86), one durum wheat variety (Oranje) and a soft biscuit wheat variety (Snack) were exposed to high temperature stress of (32 ºC/15 ºC day/ night for three days) during grain filling under controlled chamber for two consecutive seasons in comparison to non-stress conditions. On an average of two seasons, maximum reduction in starch content was observed in Snack (7.37 %) while minimum reduction observed in Kariega (1.25 %) under elevated temperatures as compared to control (**Labschage *et al*., 2009**). Previous studies revealed that filling grains is a low-oxygen organ that restricts its energy metabolism. This limited energy supply also determines the status of starch synthesis in grains. Metabolic studies confirmed that the expression of TCA cycle and ATP synthesis was significantly decreases in filling grains in response to elevated temperatures that could be one of the reasons of reduced starch content under heat stress condition (**Yamakawa and Hakata, 2010).** In an experiment the effect of terminal heat stress on carbohydrate accumulation on three wheat cultivars (BARI Gom 25, BARI Gom 26 and Pavon 76) under sown on 18 November, 2011 under two temperature conditions normal (23°C in open field) and elevated (6 ± 1°C higher than open field mean air temperature by polythene chamber) were studied. The results showed that elevation in temperature shortened the grain filling duration by 5-day in BARI Gom 25 and BARI Gom 26 and by 10-day in Pavon 76. Under elevated temperature grain starch synthesis was found to be stopped at 25days after anthesis (DAA) in Pavon 76 and at 30 days after anthesis in BARI Gom 26 (**Khatun and Ahmed 2015**). In recent studies similar trend of reduction in starch content under elevated temperature also observed. They said that the impact of high temperature in starch accumulation is usually attributed to direct effect of stress on enzymes involved in it (**Iqbal *et al.*, 2017**). Higher temperature stress impedes the synthesis of starch which is a major reservoir in wheat grain. It is due to the under-heat stress reduction in expression and activity of starch synthesis related proteins. Hence, reduced starch synthesis also been proposed to the main reason for heat stress induced reduction in grain weight. Transcriptomic analysis also concludes that the key proteins for starch synthesis including glucose 1 phosphate adenyltranferase (APGas) and granule bound starch synthetase showed significant decrease in wheat under heat stress condition as compared to non-stress condition. In an experiment, *Chinese spring* wheat kernel was evaluated to study the changes in metabolic protein under heat treatment (37/17ºC) and control condition (24 ºC/17 ºC). The results concludes that due to heat stress there was an abundant decrease in heat responsive proteins which involved in metabolism due to which metabolic process of sucrose and starch and amino acid showed a significant decrease under heat stress condition. From this study, starch accumulation, which is major component of deposition in grains of wheat was found impeded and proteomic data indicates that this may due to the protein related to starch synthesis (two starch synthases and a granule-bound starch synthetase reduced) (**Wang *et al.*, 2018**). An experiment was conducted, in which winter wheat cultivar Gaocheng 8901 was grown under controlled (25 °C/18 °C) and heat-treated condition (40°C for 2h) in growth chamber from flowering to maturity, the results show that under heat stress condition starch content was found reduced and associated with reduced thousand grain weight if wheat cultivar under elevated temperatures. Under controlled condition starch content was 76.30 % while under heat stress it reduces and become 74.50 %. Proteomics study also conclude that under heat stress starch binding enzyme IIb (SBE IIb) was found down regulated in heat stress comparison to controlled condition (**Zang *et al.*, 2018**). An experiment conducted in two wheat cultivars i.e HD2985 (thermotolerant) and HD2329 (thermo-susceptible) under two conditions i.e-controlled environment (22 ºC) and under heat stress condition (at 38 °C, 2h treatment at grain filling stage). The results concluded that through the study of functional cataloguing of stress associated active protein (SAAPs) it is concludes that the under-heat stress starch and sucrose metabolism was found altered and decrement in biosynthesis enzymes such as ADP-glucophosphorylase (1.4 fold down regulation) and soluble starch synthase (1.25 fold down regulation) was observed (**Kumar *et al.*, 2019**). A study in another crop (two hybrids of maize cultivars) was done to investigation the effect of differential stress condition on maize kernel starch accumulation. The results showed that the starch content in the wheat kernel was reduced by 42 % in drought treatment, 29 % in heat stress treatment and 58 % in combined treatment. The reasons for the decrement in starch content explained by investigator may be due to limited assimilates under stress condition or sugar-to-starch synthesis was disturbed under stress condition. Proteomics analysis also confirmed that under stress condition the expression of sucrose synthase was found reduced **(Wang *et al.*, 2020).**

4.7.2 Percentage of Amylose (%) and Amylopectin (%)

Amylose and amylopectin in seeds of each wheat varieties was observed under both sowing conditions. The percentage of amylose in all wheat varieties was found increased while percentage of amylopectin were found decreased under D2 (elevated temperatures) as compared to D1. However, the ratio of amylose to amylopectin was also found increased under D2as compared to D1. In detailed view, for amylose content under D1, highest amylose in seeds was observed in HD3086 variety (12.02 %) and lowest in HD3059(7.05 %). Under D2, amylose percent was found increased and highest value observed in HD3086 variety (20.77 %) and lowest in UP2565 (8.91 %) (**Table 4.7.2)**. Maximum percent increase in amylose content was observed in UP2967 variety (99.42 %) and minimum in UP2565 (6.23 %) (**Figure 4.7.2)**. Statistically amylose content was significantly affected by elevated temperatures in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatment × varieties were also found significant at ($p \leq 0.01$). For amylopectin, under D1, highest amylopectin in seeds was observed in HD3059 variety (92.95 %) and lowest in HD3086(87.98 %). Under D2, amylopectin was found decreased and highest value observed in UP2565 variety (91.09 %) and lowest in HD3086 (79.23 %) (**Table 4.7.2)**. Maximum percent decrease in amylopectin was observed in HD3059 variety (5.03 %) and minimum in UP2565 (0.57%) (**Figure 4.7.2)**. Statistically amylopectin content was significantly affected by D2 in terms of treatments and varieties ($p \leq 0.01$). Interaction between treatment × varieties were also found significant at ($p \leq 0.01$).

Table 4.7.2 Effect of differential temperatures on amylose (%) and amylopectin (%) in grains of different wheat varieties. *(D1 & D2 indicates sowing in the month of November and December respectively).*

	Amylose (%)		Amylopectin (%)	
Varieties	**D1**	**D2**	**D1**	**D2**
UP2628	7.61	10.22	92.39	89.78
HD3086	12.02	20.77	87.98	79.23
UP2967	8.70	17.36	91.30	82.64
UP2784	9.18	10.94	90.82	89.06
UP2526	7.15	10.53	92.85	89.47
UP2565	8.38	8.91	91.62	91.09
UP2748	8.87	12.08	91.13	87.92
HD3059	7.05	11.73	92.95	88.27

	Treatment (T)	**Variety (V)**	**(TXV)**	**Treatment (T)**	**Variety (V)**	**(TXV)**
SEm ±	0.367	0.734	1.038	0.367	0.237	0.359
CD (5%)	1.057	2.115	2.992	1.057	0.734	1.038

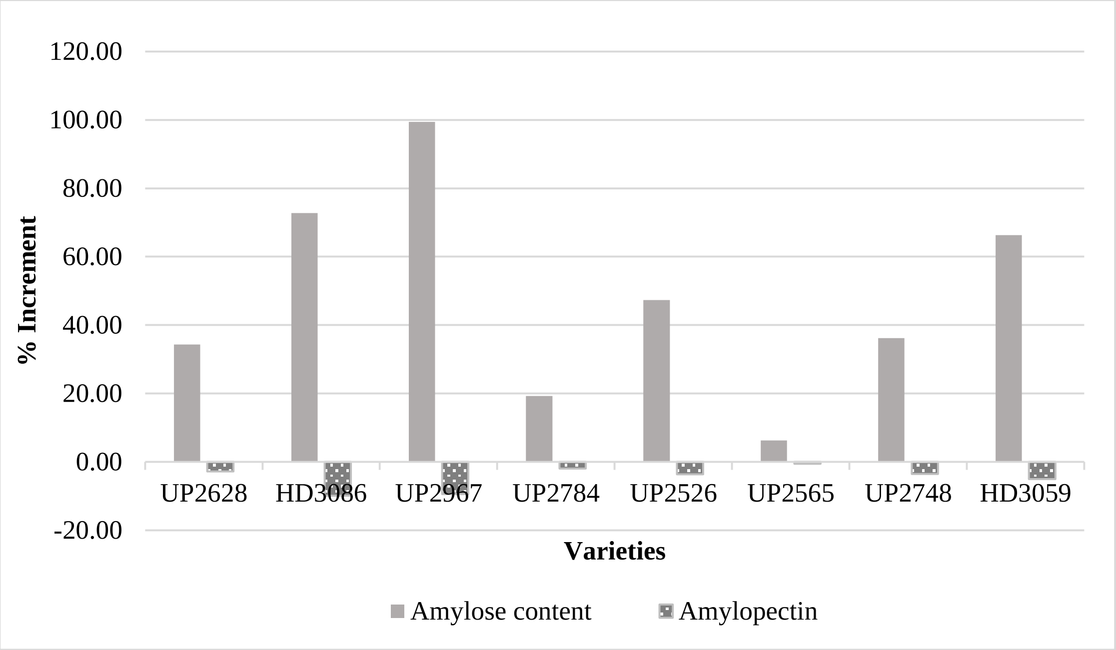

Figure 4.7.2 Percent increment in amylose content and reduction in amylopectin in grains of different wheat varieties under D2 in comparison to D1. *(D1 & D2 indicates sowing in the month of November and December respectively).*

For amylose: amylopectin ratio under D1, highest amylose: amylopectin ratio in seeds was observed in HD3086 variety (0.137) and lowest inHD3059 (0.076). Under D2, amylose: amylopectin ratio was found increased and highest value observed in HD3086 variety (0.265) and lowest in UP2565 (0.092) (**Table 4.7.3)**. Maximum percent increase in amylose: amylopectin ratio was observed in HD3086 variety (9.94 %) and minimum percent increase observed in UP2565 (0.57 %) (**Figure 4.7.3)**. Statistically ratio of amylose to amylopectin was significantly affected by D2 in terms of treatments and varieties (p≤0.01). Interaction between treatment × varieties were also found significant at (p≤0.01).

Table 4.7.3 Effect of differential temperatures on ratio of amylose amylopectin in grains of different wheat varieties. *(D1& D2 indicates sowing in the month of November and December respectively).*

	Ratio of amylose to amylopectin	
Varieties	**D1**	**D2**
UP2628	0.082	0.114
HD3086	0.137	0.265
UP2967	0.095	0.211
UP2784	0.101	0.123
UP2526	0.077	0.118
UP2565	0.091	0.092
UP2748	0.098	0.138
HD3059	0.076	0.133

	Treatment (T)	**Variety (V)**	**(TXV)**
SEm ±	0.051	0.103	0.042
CD (5%)	0.148	0.296	0.145

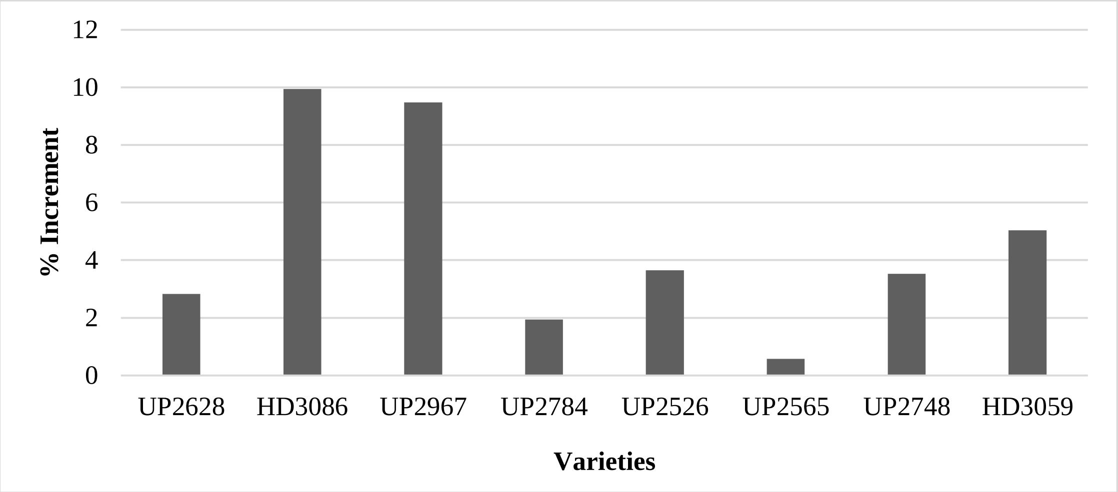

Figure 4.7.3 Percent increment in ratio of amylose to amylopectin in grains of different wheat varieties under D2 conditions. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present study, amylose content and ratio of amylose to amylopectin was found increased under elevated temperatures (D2) as compared to D1 while amylopectin was found decreased. Previous researches also reported that in wheat with increased temperature during grain filling, amylose was slightly increased and starch gelatinization temperature increased (**Shi *et al.*, 1994**). In an experiment conducted in wheat and barley it was observed that type A starch granules (amylose) starts forming about 4–7 days after anthesis and type B granules (amylopectin) appear around 10–12. When wheat and barley during grain filling were exposure to high temperatures during grain filling period (as a result of late sowing) it increases the proportions of A granules (amylose) and decrease the proportions of B granules (amylopectin) along with shortening of starch accumulation (**Dupont and Altenbach, 2003**). According another study in wheat under heat stress condition during grain filling (>30 ◦C) the amylose/ amylopectin ratio was found increases which causes a reduction in dough elasticity. The size distribution of starch granules is also modified by high temperatures (**Hurkman *et al.*, 2003**). Researches showed that the concentrations of total starch and amylopectin were markedly reduced, but amylose concentration was affected or slightly increased under elevated temperatures treatments (**Zhao *et al*. 2008).** Similar trend of significant decrement in total starch and amylopectin concentrations under elevated temperatures also observed by other researchers in which an experiment was conducted in which two bread wheat varieties (Kariega and SST86), one durum wheat variety (Oranje) and a soft biscuit wheat variety (Snack) were exposed to high temperature stress of (32 ºC/15 ºC day/ night for three days) during grain filling under controlled chamber for two consecutive seasons and the amylose content and amylose to amylopectin ratio was studied under stress conditions in comparison to non-stress conditions. The results concludes that amylose content and the amylose: amylopectin ratio was found increased under the heat treatment in comparison to control. Significant increase in amylose content under elevated temperatures was observed in the durum wheat (Oranje). On an average of two seasons, maximum increment in amylose content was observed in Snack (19.87 %) while minimum increment observed in Oranje (12.45%) under elevated temperatures as compared to control. Similarly, maximum increment in amylose:

amylopectin ratio was observed in Snack (27.78 %) while minimum increment observed in Oranje (17.35 %) under elevated temperatures as compared to control (**Labschage *et al*., 2009**). In an experiment, the effect of high temperatures (above 25 °C) on starch concentration and the morphology of starch granules in the grains of wheat (Triticum aestivum L.) cultivars Yangmai 9 and Yangmai 12 were studied under different treatments of high temperatures (25, 30, 35 and 40 °C) for 3 days at different time intervals after anthesis. The results conclude that under high temperatures, starch concentration found decreased due to the decrease of amylopectin in grains while the proportion of amylose in the total starch content found increased with increasing temperatures above 30 °C during the first 14 days, but after that it is not influenced by more elevation in temperature (**Liu *et al*., 2011**). In recent research, an experiment was conducted in two popular wheat cvs. HD3059 (thermo-tolerant) and BT-Schomburgk (thermo-susceptible) under two experimental conditions i.e optimum temperature (26/22 °C) and elevated temperatures (32 °C and 40 °C, 1 h) during grain-filling. The results concludes that under heat stress in both cultivars an increase in the amylose content was observed. Amylose content found maximum in BT-Schomburgk (~ 12%) under HS (40 ºC, 1 h). However, much difference in amylose content was not seen between the cultivars but significant difference was observed in amylopectin Content which was found decrease due to elevation in temperature. Lower amylopectin content was observed under HS (40 ºC, 1 h) inHD3059 (~ 41 %) and in BT-Schomburgk (~ 31 %) compared to control condition. According to the author, at high temperatures, decrease in amylopectin content is may be due to reduced starch content under heat stress condition and decrease in amylolytic activity under stress condition (**Kumari *et al*., 2020**).

4.7.4 Percentage of Grain Nitrogen (%) and Total soluble protein (%)

Grain nitrogen and total soluble protein in grains of each wheat varieties was observed under both sowing conditions. Grain nitrogen and total soluble protein in all wheat varieties was found increased under D2 (elevated temperatures) as compared to D1. In detailed view, for grain nitrogen, under D1, highest grain nitrogen was observed in UP2967 variety (0.30 %) and lowest in UP2526 (0.13 %) while under D2, grain nitrogen was found increased and highest value observed in HD3086 variety (0.45 %) and lowest in UP2526 (0.20 %) (**Table 4.7.4)**. Maximum percent increase in grain nitrogen was observed in UP2565 variety (86.79 %) and minimum in UP2628 (26.23 %) (**Figure 4.7.4)**. Statistically grain nitrogen was significantly and positively affected by elevated temperatures in terms of treatments and varieties (p≤0.01). Interaction between treatment × varieties were found non-significant at (p>0.05). For total soluble protein under D1, highest protein in seeds was observed in UP2967 variety (10.72 %) and lowest in UP2748 (7.72 %) while under D2, total protein was found increased and highest value observed in UP2628 and UP2967 variety (12.40 %) and lowest in UP2784 (10.23 %) (**Table 4.7.4)**. Maximum percent increase in total soluble protein was observed in UP2565 and UP2748 variety (41.46 % and 45.42 %) and minimum in UP2526 (19.11 %) (**Figure 4.7.4)**. Statistically total soluble protein was significantly affected by D2in terms of treatments and varieties (p≤0.01). Interaction between treatment × varieties were also found significant at (p≤0.01).

Table 4.7.4 Effect of differential temperatures on grain nitrogen (%) and total soluble protein (%) in grains of different wheat varieties. *(D1 & D2 indicates sowing in the month of November and December respectively).*

<table>
<tr><th colspan="5">Grain nitrogen (%)</th><th colspan="4">Protein Content (%)</th></tr>
<tr><th></th><th colspan="2">D1</th><th colspan="2">D2</th><th colspan="2">D1</th><th colspan="2">D2</th></tr>
<tr><td>UP2628</td><td colspan="2">0.17</td><td colspan="2">0.21</td><td colspan="2">9.58</td><td colspan="2">12.40</td></tr>
<tr><td>HD3086</td><td colspan="2">0.29</td><td colspan="2">0.45</td><td colspan="2">8.74</td><td colspan="2">11.63</td></tr>
<tr><td>UP2967</td><td colspan="2">0.30</td><td colspan="2">0.41</td><td colspan="2">10.72</td><td colspan="2">12.40</td></tr>
<tr><td>UP2784</td><td colspan="2">0.16</td><td colspan="2">0.27</td><td colspan="2">8.50</td><td colspan="2">10.23</td></tr>
<tr><td>UP2526</td><td colspan="2">0.13</td><td colspan="2">0.20</td><td colspan="2">9.63</td><td colspan="2">11.47</td></tr>
<tr><td>UP2565</td><td colspan="2">0.18</td><td colspan="2">0.33</td><td colspan="2">8.69</td><td colspan="2">12.31</td></tr>
<tr><td>UP2748</td><td colspan="2">0.15</td><td colspan="2">0.22</td><td colspan="2">7.72</td><td colspan="2">11.23</td></tr>
<tr><td>HD3059</td><td colspan="2">0.16</td><td colspan="2">0.24</td><td colspan="2">7.86</td><td colspan="2">10.91</td></tr>
<tr><td></td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td><td>Treatment (T)</td><td colspan="2">Variety (V)</td><td>(TXV)</td></tr>
<tr><td>SEm ±</td><td>0.113</td><td colspan="2">0.227</td><td>0.321</td><td>0.059</td><td colspan="2">0.041</td><td>0.055</td></tr>
<tr><td>CD(5%)</td><td>0.327</td><td colspan="2">0.655</td><td>0.926</td><td>0.172</td><td colspan="2">0.119</td><td>0.169</td></tr>
</table>

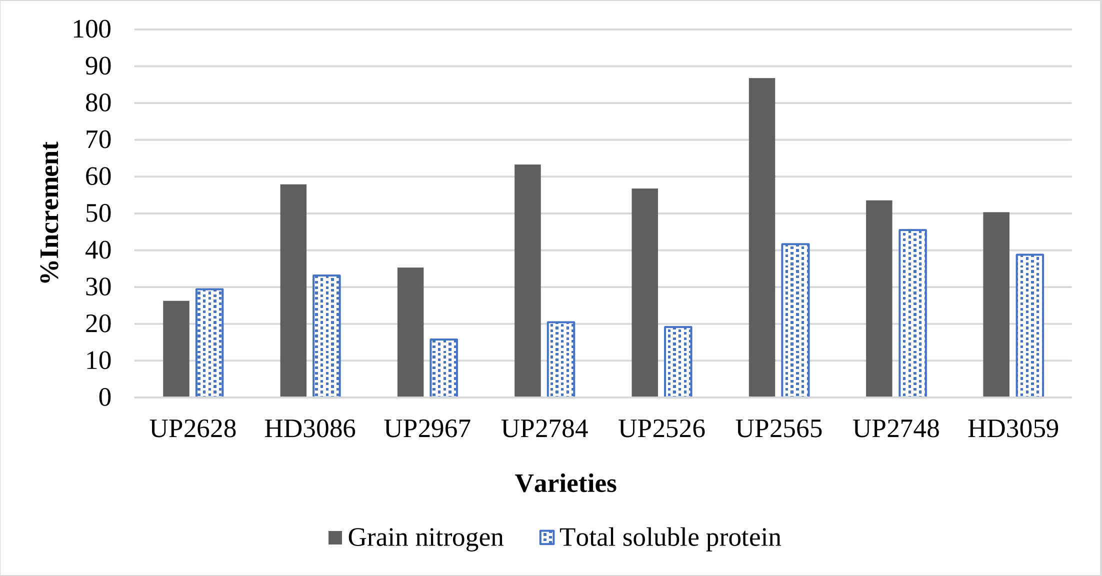

Figure 4.7.4 Percent increment in grain nitrogen and total soluble protein in grains of different wheat varieties under D2 conditions. *(D1& D2 indicates sowing in the month of November and December respectively).*

In present study, grain nitrogen content (%) found increased under D2 as compared to D1. Nitrogen is required by wheat grains for synthesis of proteins during kernel development. Similar results also observed by some previous authors in which heat stress increases the grain nitrogen content in wheat (*T. aestivum* L.) **(Bhullar and Jenner 1985, Viswanathan and Khanna-Chopra 2001).** An experiment was conducted in which the effect of heat stress on grain nitrogen content was analysed in different wheat (Triticum aestivum L.) varieties PBW154, Sonalika and Hindi62 under three sowing dates (19 November, 14 December and 18 January). The results concludes that the grain nitrogen content at maturity was found increased in both varieties under heat stress condition. Elevation in temperatures causes increased the nitrogen content in both varieties by 32 % in PBW154 and 41 % inHindi62. This increase in the grain nitrogen percentage, according to the author could be due to the decrease in starch accumulation and an increase in protein synthesis (**Viswanathan and Khanna-Chopra, 2001).** Under elevated temperatures or heat stress protein synthesis in cereal crops such as in wheat found reduced but storage protein such as gliadins and heat shock proteins found increased as a result nitrogen content per storage protein also found increased under elevated temperature conditions (**Trebst *et***

***al.,* 2002**). Nitrogen concentration in cereals such as wheat grains are the result of significant remobilization of nitrogen from leaves, decrease in lead nitrogen under stress condition such as heat stress and forced senescence under delay in sowing conditions also affects the amount of nitrogen available for remobilization of nitrogen into grains (**Taub *et al*., 2008**). During heat stress, grain filing phases reduced and reduction in starch deposition can easily observed. Due to decreased starch accumulation, it influences protein concentration in wheat kernel by allowing more nitrogen per unit starch in grains. Although, daily flow of carbon and nitrogen into grain increases with increasing temperatures however, carbon flow decreases per degree-day as a result grain size found more affected under heat stress than quantity of nitrogen content in grains (**Farooq *et al*., 2011).** According to a review in wheat it concluded that during heat stress grain filling duration decreases, it affects the grain dry matter and nitrogen accumulation. So as the grain nitrogen increases (**Nuttall *et al*., 2017**).

In present study, total protein (%) found increased under D2 as compared to D1. Previous reports observed that under elevated temperature grain protein percentage was enhanced while the protein content per grain was reduced. As under heat stress condition, formation of more storage proteins and heat shock proteins increases as a result protein percent also increases but functional proteins related to enzymes and enzyme functionality found disturbed due to heat stress this leads to reduction in protein content per grain observed. So,it can be said that under elevated temperatures total protein percentage found increased (**Krishnan *et al*., 1989).** In wheat grain, the major component of non-starchy content is grain protein. During an experiment, 32 wild and cultivated genotypes of wheat species belonging to diploid (donors may be of B, A and D genomes), tetraploid (BBAA and AAGG genomes) and hexaploid (BBAADD genome) were evaluated for heat stress tolerance under two dates i.e 18 November (normal sowing) and 15 January (heat stress). The results concludes that under heat stress the non-starch content of the grain (total protein) showed a significant increase in hexaploids (1.3 mg grain^{-1}), while in tetraploids and diploids it did not increase (**Chinnusamy and Chopra, 2003**). Grain protein increases under heat stress but functionality of protein significantly reduced affecting the overall grain quality. Heat stress also decreases the duration but not the rate of protein deposition in grains. The greatest increase in grain protein in wheat occurs when heat stress is imposed early in grain filling (**Castro *et al.,* 2007**). In an experiment, two bread wheat varieties (Kariega and SST86), one durum wheat variety (Oranje) and a soft biscuit wheat variety (Snack) were exposed to high temperature stress of (32 ºC/15 ºC day/ night for three days) during grain filling under controlled chamber for two consecutive seasons and the grain protein was studied in comparison with non-stress condition. The results concludes that heat treatment significantly increased protein content in all wheat varieties however, the flour protein content was found significant in one bread wheat (Kariega) under heat stress as compared to non-stress condition. On comparing between different wheat varieties, flour protein content was significantly higher for the durum wheat and soft wheat as compared to the bread wheats. This increase in protein was mainly due to an increase in the soluble (gliadin) protein in response to elevated temperatures. On an average of two seasons, maximum increment in protein content was observed in Oranje (10.58%) while minimum increment observed in SST86 (3.87%) under elevated temperatures as compared to control. In contrast, wheat variety Kariega showed decrement in protein content during second season under heat stress condition as compared to control but on an average the decrement was found insignificant (0.60 % decrease) (**Labschage *et al.,* 2009**). In another experiment significance of heat stress response and expression of heat shock proteins in thermo-tolerance of cereal yield (wheat) and quality has been observed. The results concludes that high temperature during grain filling activates heat shock genes causing the mature grain to contain more protein but this accumulated protein provide thermo-tolerance to grain & produce weaker dough. According to the research, heat shock proteins in wheat grains starts synthesizing at 34°C and disappeared after shifting of seedlings to 22ºC. The synthesis of HSPs was responsible for acquisition of thermo-tolerance for seedling to survive. (**Rampino *et al.,* 2009**). An experiment was conducted to study the effect of three planting dates; 25th October, 25th November and 25th December on grain quality of four bread wheat (*Triticum aestivum* L.) cultivars; Shandaweel1, Sids 12, Giza 168 and Sakha 93, during the two successive growing seasons of 2012/2013 and 2013/2014. The results showed that delay in sowing month adversely affected the carbohydrate %while it increased the grain protein % in all four wheat cultivars under stress condition (**El-Maghraby *et al*., 2016**). An experiment was conducted in which winter wheat cultivar Gaocheng 8901 was grown under controlled (25°C/18°C) and heat-treated condition (40°C for 2h) in growth chamber from flowering to maturity, the results show that under heat stress condition protein content was found increased. Under controlled condition, protein content was 14% in grains of wheat cultivar while under heat stress it increased by 2% and becomes 16% (**Zang *et al*., 2018**). In a two-year experiment, *Chinese spring* wheat was evaluated for total protein content under heat treatment (37/17ºC) and control condition (24ºC/17ºC). The results concludes that due to heat stress there was an increase of 32.8% and 23.6% in total protein content during two consecutive years of 2015-16 and 2016-17 under stress condition as compared to controlled condition. This significant increase was due to the increase in stress responsive protein (HSP70, HSP40, HSP20, redox responsive proteins) and storage protein under elevated temperatures in wheat kernel (**Wang *et al*., 2018**). An experiment conducted in two wheat cultivars i.e HD2985 (thermotolerant) and HD2329 (thermo-susceptible) under two conditions i.e-controlled environment (22ºC) and under heat stress condition (at 38 °C, 2h treatment at grain filling stage). The results concluded that under heat stress condition there was an increase in protein concentration which is called stress associated active proteins (SAAPs) in both cultivars. This shows the general trend of increasing protein concentration under elevated temperatures. On comparing between cultivars, about 1.6 folds increase in expression of SAAPs observed in heat treated HD2985 as compared to heat treated HD2329 (**Kumar *et al*., 2019**). In an experiment, *Chinese spring* wheat kernel was evaluated to study the increase in storage protein under heat treatment (37/17ºC) as compared to control condition (24ºC/17ºC). The results concludes that due to heat stress, 10 heat responsive protein (HRPs) were found which belong to storage proteins and were also significantly enriched among increased protein. A new storage protein called cupin protein which is similar to another wheat storage protein globulin 3 (80% identical) was also observed. From this finding it is also conclude that this cupin protein accumulation could be the reason of relative increase in protein content in heat stressed wheat grain (**Wang et al., 2018**). A significant increase in total protein (11.5 to 13.5%) content in grains of 17 Indian wheat genotypes was reported when they sow late (12th December 2016) as compared to normal (15th November). While highest increase in protein content was observed in variety CL3949 48% due to delayed sowing **(Singh *et al*., 2020).**

Correlation analysis between seed quality parameters with grain yield under both sowing condition

Correlation was done between grain yield and seed quality parameters under both sowing conditions. The results showed that, grain yield was positively correlated with all the parameters of seed quality under D1 while under D2, negative correlation was found between grain yield and 4 parameters i.e carbohydrate content, starch content, amylose content and with grain nitrogen. From this it is clear that under D2, grain quality of wheat is badly affected due to elevated temperature. Under D1, significant and positive correlation was observed between carbohydrates and starch (r=0.99**) while negative but significant correlation was observed between amylose and amylopectin (r=-0.99**). Under D2, condition significant and positive correlation was observed between carbohydrates and starch (r= 0.99**) while negative correlation was observed between amylose and amylopectin (r=-0.99**), amylopectin and grain nitrogen (r=-0.82) (**Table 4.7.5.1 and Table 4.7.5.2).**

Table 4.7.5.1. Pearson's correlation between seed quality parameters with grain yield under D1.

	GY	*CHO*	*ST*	*AMY*	*AMYL*	*PR*	*GN*
GY	1						
CHO	0.05	1					
ST	0.05	0.99**	1				
AMY	0.09	0.25	0.25	1			
AMYL	-0.09	-0.19	-0.19	-0.99**	1		
PR	0.29	0.56	0.56	-0.09	0.10	1	
GN	0.24	0.70	0.70	0.65	-0.64	0.49	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Carbohydrate content (CHO), Starch Content (ST), Amylose content (AMY), Amylopectin (AMYL), Total soluble Protein (PR) and Grain Nitrogen (TSW).

Table 4.7.5.2 Pearson's correlation between seed quality parameters with grain yield under D2.

	GY	*CHO*	*ST*	*AMY*	*AMYL*	*PR*	*GN*
GY	1						
CHO	-0.66	1					
ST	-0.66	0.99**	1				
AMY	-0.50	-0.09	-0.09	1			
AMYL	0.50	0.09	0.09	-0.99**	1		
PR	0.37	-0.40	-0.40	0.13	-0.13	1	
GN	-0.35	-0.37	-0.37	0.82*	-0.82*	0.320186	1

***. Correlation is significant at the 0.01 level (2-tailed),*. Correlation is significant at the 0.05 level (2-tailed).*

Note; Grain yield (GY), Carbohydrate content (CHO), Starch Content (ST), Amylose content (AMY), Amylopectin (AMYL), Total soluble Protein (PR) and Grain Nitrogen (TSW).

4.8 Seed Protein Profiling of selected wheat varieties sown at different time intervals

On the basis of grain yield analysis and heat susceptibility index of eight wheat varieties, mature seeds of two varieties (UP2784 and UP2748) were selected for comparison of storage protein banding patterns through SDS page analysis. The UP2784 variety with highest heat susceptibility index (1.48) and highest reduction in grain yield (20.05 %) under D2 was found highly sensitive towards elevated temperatures while UP2748 variety with lowest heat susceptibility index (0.38) and least reduction in grain yield (2.07 %) under D2 considered as tolerant towards heat stress **(Table 4.3.7 and Figure 4.3.5).**

During investigation it was observed that storage proteins (albumin, globulin, gliadian and gluten was increased under D2 as compared to D1 in both wheat cultivars. On comparing both the wheat varieties, higher storage protein accumulation was observed in heat tolerant wheat variety (UP2748) as compared to heat sensitive variety (UP2784) **(Table 4.8.1).** The increment of 334.62 % in albumin, 48.94% in globulin, 18.30 % in gliadin and 28.33 % in gluten was found in variety UP2784 under D2as compared to D1 while variety UP2748 showed 152.42 % increment in albumin, 16.19 % in globulin, 113.12 % in gliadin and 104.60 % in gluten under D2 as compared to D1 (**Figure 4.8.1).** Statistically values of storage protein albumin was significantly increased by elevated temperatures and significant difference was observed between treatments and varieties (p≤0.01) however, the interactions between treatments × varieties were found non-significant at (p>0.05). Globulin protein was significantly increased by elevated temperatures and significant difference was observed between treatments at (p≤0.05) and varieties at (p≤0.01) however, the interactions between treatments × varieties were found non-significant at (p>0.05). Similarly, gliadin protein was significantly increased by elevated temperatures and significant difference was observed between treatments at (p≤0.05) while non-significant difference was observed between varieties (p>0.05). However, the interactions between treatments × varieties were also found significant at (p≤0.05). While in gluten protein, the increment in values of gluten protein under elevated temperatures was found non-significant as compared to non-stressed conditions at (p>0.05) along with that there was non-significant difference was observed between treatments, varieties as well as the interaction between treatments ×varieties at (p>0.05).

Table 4.8.1 Effect of differential temperatures on wheat kernel storage proteins. *(D1& D2 indicates sowing in the month of November and December respectively).*

	D1				**D2**			
	Albumin (%)	**Globulin (%)**	**Gliadin (%)**	**Gluten (%)**	**Albumin (%)**	**Globulin (%)**	**Gliadin (%)**	**Gluten (%)**
UP2784	1.76	0.40	2.68	6.48	7.65	0.59	3.17	8.32
UP2748	3.50	2.09	2.39	4.42	8.84	2.43	5.08	9.04
		Treatment (T)		**Variety (V)**		**(TXV)**		
Albumin	**SEm ±**	0.159		0.159		0.225		
	CD (5%)	0.519		0.519		0.734		
Globulin	**SEm ±**	0.754		0.754		0.106		
	CD (5%)	0.245		0.245		0.347		
Gliadin	**SEm ±**	0.282		0.282		0.399		
	CD (5%)	0.921		0.921		1.303		
Gluten	**SEm ±**	1.059		1.059		1.498		
	CD (5%)	3.454		3.454		4.885		

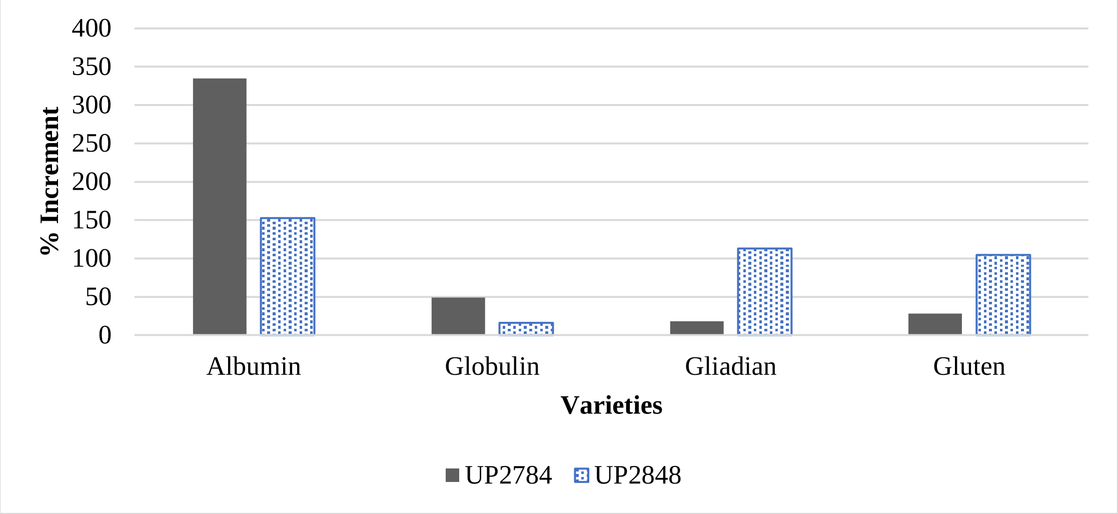

Figure 4.8.1 Percent increment in wheat kernel storage protein under D2 as compared to D1 condition. *(D1 & D2 indicates sowing in the month of November and December respectively).*

a) Total soluble Proteins

Total proteins in grains of both the wheat varieties UP2784 (heat-sensitive) and UP2748 (heat-tolerant) were analysed through SDS PAGE. A band of molecular weight 14.30 KDa with dark intensity was observed in heat tolerant variety under both sowing condition (Rf = 0.835 and 0.852 under D1 and D2 respectively) and in heat-sensitive variety under D2 (Rf = 0.83) while this band was found absent in heat-sensitive variety under D1. From this it can be concluded that the absence of 14.30 KDa molecular weight protein responsible for heat sensitive behaviour while its presence provides information that particular variety is experiencing heat stress. Presence of 14.30 KDa (Rf= 0.835) molecular weight protein in heat tolerant variety even under D1 indicates that this variety has the proteins that is responsible for its characteristic tolerant behaviour. On comparing the wheat varieties according to their sowing conditions, it can be observed that a band of 43.00 (Rf = 0.339 KDa) with very light intensity was present in heat-sensitive variety under D1 while it was found absent under D2. From this information it can be concluded that the proteins near the molecular weight 43.00 KDa found highly sensitive towards elevated temperatures as they may be disrupted due to heat stress. Under D2, bands of molecular weight 14.30 KDa (Rf = 0.831) (dark intensity) and 20.00 KDa (Rf = 0.693) (very-very light intensity) were observed and found absent under D1 in heat sensitive wheat variety. Along with that a clear separation of peptide bands between 20.00 KDa to 29.00 KDa was also observed under D2 in same variety which was found less clear under D1. From this it can be concluded that, these molecular weight proteins could be responsible for the sensitive behaviour of UP2784 (heat sensitive) under D2 as these molecular weight proteins were found absent in same variety under D1 conditions. In tolerant wheat variety, bands of 14.30 KDa (Rf = 0.859) and 43.00 KDa (Rf = 0.318) with dark intensity was observed under D2 conditions which was found absent under D1. Dark intensity of these bands in tolerant wheat variety could be the reason for tolerant nature of that particular variety under D2 condition (**Figure 4.8.2)**

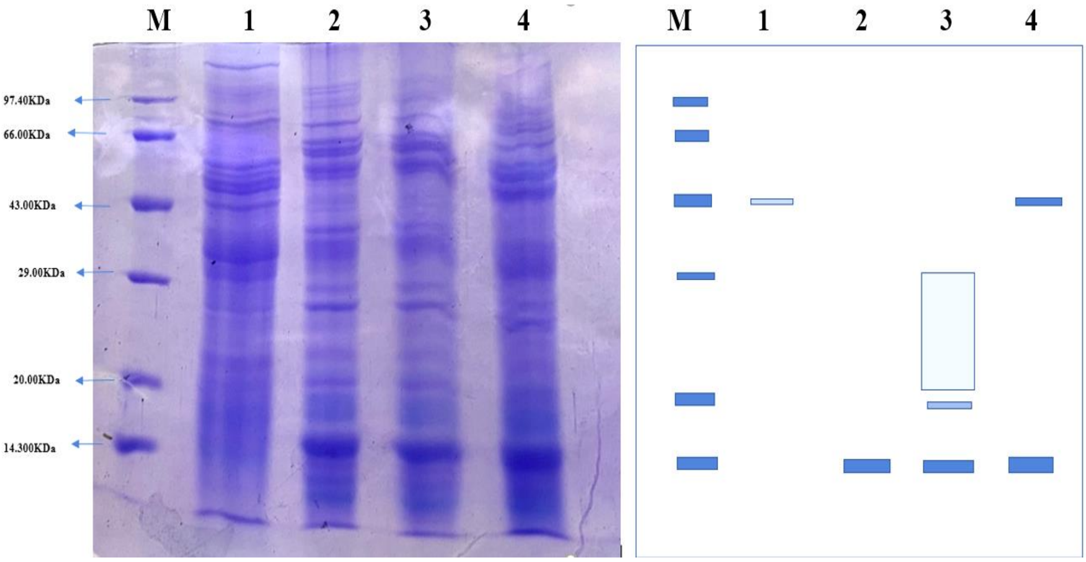

Figure 4.8.2 SDS PAGE profile of total soluble proteins in grains of selected wheat varieties UP2784 and UP2748 under D1 and D2 sowing conditions.

Lane M; 14 to 100 KDa Marker, Lane 1: UP2784 (D1), Lane 2: UP2748 (D1), Lane 3: UP2784 (D2), and Lane 4: UP2748 (D2).

Protein Marker Rf value; **97.40 KDa** = 0.123, **66.00 KDa** = 0.203, **43.00 KDa** = 0.345, **29.00 KDa** = 0.498, **20.00 KDa** = 0.723, **14.30 KDa** = 0.865

(Note- Dark colour boxes indicate dark intensity of bands, light colour boxes indicate light intensity of bands, long box indicates continuous separation of bands).*

b) Albumins

The protein profiling of albumins in grains of both the varieties UP2784 (heat-sensitive) and UP2748 (heat-tolerant) was also analysed with SDS page and it was observed that the number and the intensity of bands (darker) were more in both varieties under D2 as compared to D1. This was may due to the increased albumin content under D2 as compared to D1 in both wheat varieties. Peptides of molecular weight 29.00 KDa (for heat tolerant; Rf = 0.586, for heat sensitive Rf = 0.585) and near 66.00 KDa (for heat tolerant; Rf = 0.243, for heat sensitive Rf = 0.229) were found highly expressed under D2 in both varieties as compared to D1 in which they were found faded. Similarly, bands of 14.30 KDa (for heat tolerant; Rf = 0.846, for heat sensitive Rf = 0.866) and near 20 KDa (for heat tolerant; Rf = 0.723, for heat sensitive Rf = 0.731) were found more intense and darker in the both varieties under D2 in comparison to D1. A band nearer to 97.40 KDa (for heat tolerant; Rf = 0.145, for heat sensitive Rf = 0.131) (very-very light intensity) under D2 in both wheat varieties also observed which was absent under D1. Presence of various molecular weight proteins in both the varieties under D2 signifies that elevating temperature due to the D2 significantly increased various heat shock proteins, responsible for more accumulation of storage protein albumin under stress condition (**Figure 4.8.3).**

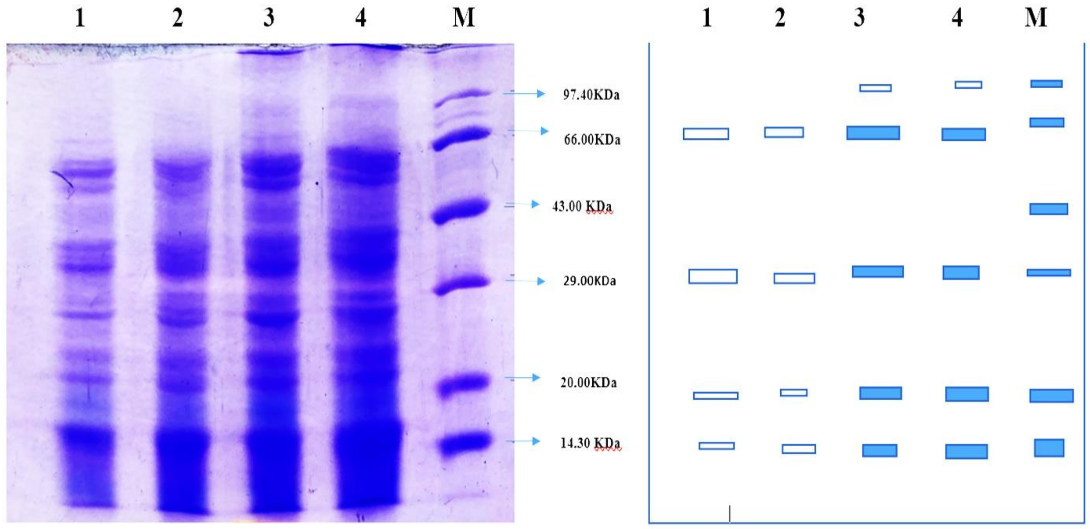

Figure 4.8.3 SDS PAGE profile of storage protein albumins in grains of selected wheat varieties UP2784 and UP2748 under D1 and D2 sowing conditions.

Lane 1; UP2748 (D1), Lane 2: UP2784 (D1), Lane 3: UP2748 (D2), and Lane 4: UP2784 (D2), Lane M: 14 to 100 KDa Marker.

Protein Marker Rf value; **97.40 KDa** = 0.103, **66.00 KDa** = 0.197, **43.00 KDa** = 0.355, **29.00 KDa** = 0.527, **20.00 KDa** = 0.843, **14.30 KDa** = 0.872

(Note- (Dark colour boxes indicates dark intensity of bands, light colour boxes indicate light intensity of bands, long box indicates continuous separation of bands).*

c) Globulin

Protein profiling of globulin protein in grains of both varieties UP2784 (heat-sensitive) and UP2748 (heat-tolerant) indicate that no big difference in banding patterns in both varieties under two different sowing conditions. However, under D2, number of bands and their intensity was found slightly higher in comparison to D1. In heat-sensitive variety, peptide bands of molecular weight between 29.00 KDa to 43.00 KDa were found lighter under D1 and darker under D2. However, these band were absent in heat-tolerant variety under both sowing conditions. Although heat-tolerant variety had more banding patterns near 66.00 KDa under D2 as compared to same variety under D1 which could be the reason for tolerant behaviour of that variety under heat stress condition (**Figure 4.8.4).**

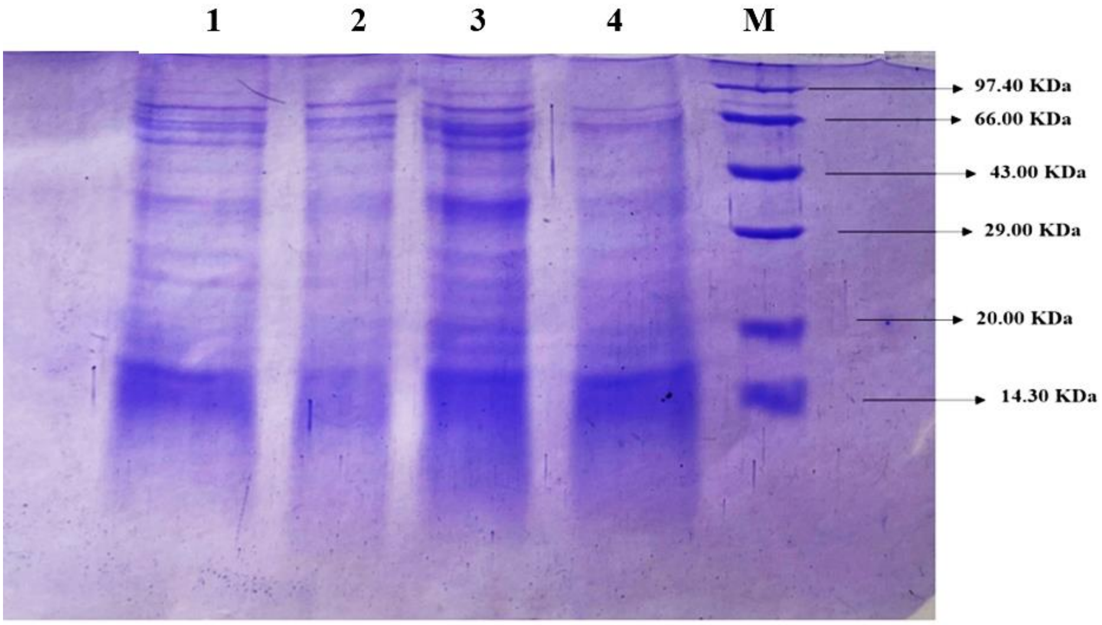

Figure 4.8.4 SDS PAGE profile of storage protein globulin in grains of selected wheat varieties UP2784 and UP2748 under D1 and D2 sowing conditions.

Lane 1; UP2748 (D2), Lane 2: UP2784 (D2), Lane 3: UP2748 (D1), and Lane 4: UP2784 (D1), Lane M: 14 to 100 KDa Marker.

Protein Marker Rf value; **97.40 KDa** = 0.088, **66.00 KDa** = 0.142, **43.00 KDa** = 0.254, **29.00 KDa** = 0.373, **20.00 KDa** = 0.574, **14.30 KDa** = 0.712

(Note- (Dark colour boxes indicates dark intensity of bands, light colour boxes indicate light intensity of bands, long box indicates continuous separation of bands).*

d) Gliadin

Protein profiling of gliadin in grains of both the wheat varieties UP2784 (heat-sensitive) and UP2748 (heat-tolerant) indicated that not much difference was observed in banding patterns of wheat varieties under D2 in comparison to D1. No bands were observed between 29.00KDa to 20.00 KDa, 20.00 KDa to 14.30 KDa and above 66 KDa in both varieties under both sowing conditions. Maximum number of bands observed in between 29.00 KDa to 43.00 KDa. A single band of 43.00 KDa was observed in all wheat varieties Under D1; for heat sensitive, Rf = 0.312, for heat tolerant (0.321) and under D2; for heat sensitive, Rf = 0.328 for heat tolerant (0.321). It was found darker and intense for heat-sensitive variety under D1 and was lighter for heat-tolerant variety under both sowing conditions. From this it can be concluded that 43.30 KDa protein was heat sensitive. Overall, much faded and light bands observed for heat-sensitive wheat variety under D2 condition as compared to D1 (**Figure 4.8.5).**

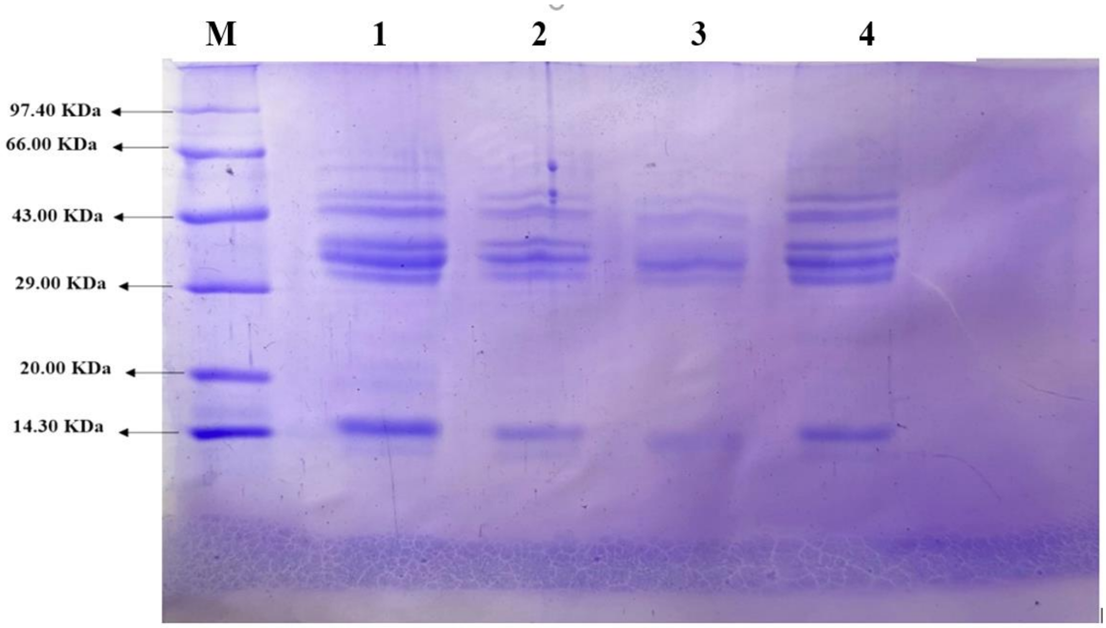

Figure 4.8.5 SDS PAGE profile of storage protein gliadin in grains of selected wheat varieties UP2784 and UP2748 under D1 and D2 sowing conditions.

Lane M; 14 to 100 KDa Marker, Lane 1: UP2784 (D1), Lane 2: UP2748 (D1), Lane 3: UP2784 (D2), and Lane 4: UP2748 (D2).

Protein Marker Rf value; **97.40 KDa** = 0.104, **66.00 KDa** = 0.200, **43.00 KDa** = 0.333, **29.00 KDa** = 0.483, **20.00 KDa** = 0.661, **14.30 KDa** = 0.814

(Note- (Dark colour boxes indicates dark intensity of bands, light colour boxes indicate light intensity of bands, long box indicates continuous separation of bands).*

e) Gluten

Protein profiling of gluten protein in grains of wheat varieties UP2784 (heat-sensitive) and UP2748 (heat-tolerant) indicated that a greater number of bands and darker bands appears in both wheat varieties under D2 as compared to D1 as gluten was found positively affected by elevated temperatures. Peptide band near 20.00 KDa and 66.00 KDa was found lighter under D1 in both wheat variety while under D2 it was found darker. This is may be the accumulation of molecular weight proteins (near 20.00 KDa and 66.00 KDa) were increased under D2 as compared to D1. For band near to 20.00 KDa, under D1, for heat sensitive variety Rf = 0.760 while for tolerant, Rf = 0.817. Under D2, for heat sensitive variety Rf = 0.745 while for tolerant, Rf = 0.790. Similarly, For band of 66.00 KDa, under D1, for heat sensitive variety Rf = 0.293 while for tolerant, Rf = 0.174. Under D2, for heat sensitive variety Rf = 0.109 while for tolerant, Rf = 0.181.Out of all storage proteins profiles maximum banding patterns was observed in storage protein gluten as compared to albumin, globulin and gliadin while minimum number of bands observed for storage protein gliadin. This could be due to the gluten protein increased under elevated temperature while gliadin least so banding patterns were found more in gluten as compared to gliadin (**Figure 4.8.6.)**

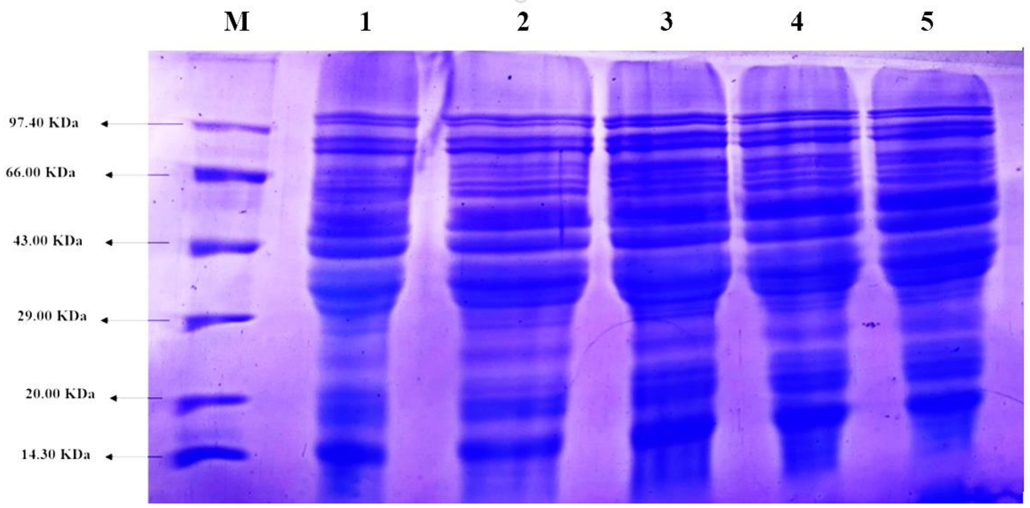

Figure 4.8.6 **SDS PAGE profile of storage protein gluten in grains of selected wheat varieties UP2784 and UP2748 under D1 and D2 sowing conditions.**

Lane M; 14 to 100 KDa Marker, Lane 1: UP2784 (D1), Lane 2: UP2748 (D1), Lane 3: UP2784 (D2), Lane 4: UP2748 (D2), Lane 5: UP2748 (D2).
Protein Marker Rf value; **97.40 KDa** = 0.150, **66.00 KDa** = 0.267, **43.00 KDa** = 0.442, **29.00 KDa** = 0.587 **20.00 KDa** = 0.760, **14.30 KDa** = 0.883
(Note- (Dark colour boxes indicates dark intensity of bands, light colour boxes indicate light intensity of bands, long box indicates continuous separation of bands).*

During investigation expression of storage protein was increased under elevated temperatures (D2) as compared to non-stress (D1). In a related study an experiment was conducted to study the effect of heat stress on heat-responsive proteins in wheat endosperm (*Triticum aestivum* cv. Thésée), plants were grown under field conditions and then transferred to a tunnel after flowering, subjected to two thermal treatments i.e 18°C for control and 34°C for treated plants. Under elevated temperatures various proteins of different molecular weight were upregulated as compared to controlled. Proteins belonging to the family of 20 kDa considered as small HSPs (HSP20) were found upregulated under heat stress. Small heat shock proteins (sHSPs) are the most prominent heat-induced proteins in plants. Along with that other heat shock proteins of molecular mass within range of 15–40 kDa was also found upregulated under stress condition. Some proteins of different molecular masses such as small heat shock protein of molecular weight (26.58 kDa), Vacuolar ATP synthase subunit E (26.47 kDa), GTP-binding nuclear protein RAN 1B (24.34 kDa), Heat shock 22 protein (23.59 kDa), Eucaryotic translation initiation factor 4E (23.52 kDa) 17 kDa class II heat shock protein (17.13 kDa) was found upregulated while Adenosyl-methionine synthetase (43.7 kDa) was downregulated under heat stress as compared to control (**Majoul *et al.*, 2003***)*.In order to check the effect of sowing condition on protein profiling through SDS PAGE an experiment was performed with 12 different wheat genotypes under two sowing conditions normal/elevated temperatures. The results concludes that the banding pattern and the intensity of peptide bands was found increased under elevated temperatures as compared to normal conditions. One extra band of 97.40 KDa was expressed in elevated temperatures condition in some wheat genotypes i.e K-0307, HI 1544, DBW 14 and Raj 3765, HW5021, PBW 574 and HI 1539, may be a band of heat shock protein (**Dhyani, 2010).** The *Chinese spring* wheat kernel was also evaluated for expression of storage protein under heat treatment (37/17ºC) as compared to control condition (24ºC/17ºC). Under heat exposure, 10 heat responsive protein (HRPs) were found which belong to storage proteins and were also significantly enriched among increased protein. A new storage protein, cupin which is similar to another wheat storage protein globulin 3 (80% identical) was also reported. It may be concluding here that cupin protein accumulation could be the reason of relative increase in protein content in heat stressed wheat grain. From SDS page analysis several bands around 70 kD were found to be increased in heat stress treatment. The bands around 70kD showed consistence increased in matured heat stressed grains (**Wang *et al*., 2018**). In an experiment, winter wheat cultivar Gaocheng 8901 was grown as controlled (25°C/18°C). Heat treated condition (40°C for 2h) was given in growth chamber from flowering to maturity, the results showed that under heat stress condition, grains become narrow, reduced weight while length remain unchanged. A significant increase in storage proteins like gluten, gliadin, globulin and albumin was found under stress condition as compared to control. High temperature stress leads to changes in expression of grain storage protein. Proteomics studies revealed that two storage protein, 12S globulin and 19KDa globulin were upregulated under high temperatures, seven gliadin proteins were differently expressed under high temperatures in which two gliadin (alpha and gama) were upregulated (**Zang *et al*., 2018**). Two wheat cultivars i.e HD2985 (thermotolerant) and HD2329 (thermo-susceptible) under two conditions i.e-controlled environment (22ºC) and under heat stress condition (at 38 °C, 2h treatment at grain filling stage) were evaluated. The results concluded that under heat stress condition there was 3.7fold increase in expression gamma gliadian seed storage protein in HD2985 and 2.3-fold downregulation in HD2329 under stress condition. This variation is may be due to diversity in heat tolerance capacity of different wheat germplasm. Similarly, in thermotolerant wheat cultivar (HD2985), 1.42fold increase in Globilin 3B and 1.33fold increase in Globulin -1 allele storage protein was observed in comparison to similar cultivar under controlled condition while in thermo-susceptible cultivar (HD2329), 0.40- and 0.56-fold increase in Globilin 3B and Globulin -1 allele was observed in comparison to similar cultivar under controlled condition (**Kumar *et al*., 2019**). A wheat variety “Rete nazionaleduro” were grown under different temperatures conclude that higher temperature during grain filling, causes increase in low molecular weight glutenins, along with increasing gliadins content in wheat kernel (**Graziano *et al*., 2020).** The SDS-PAGE analysis of total soluble seed storage proteins in 16 Indian wheat varieties concludes that under elevated temperatures conditions, increased amount of different molecular weight peptides was observed in comparison to non-stress conditions. Several polypeptide bands ranging from 11 KDa to 129 KDa was observed under elevated temperatures which was found absent under non-stressed conditions. Majority of the wheat varieties showed higher accumulation of 122KDa peptide proteins under elevated temperatures. Accumulation of 84 KDa polypeptide was found higher under late sowing. Glutenin Protein (29KDa to 43KDa) were found highly influenced by heat stress and the level of gliadins found increased under elevated temperatures. This study indicates the accumulation of major gliadins and glutenins was directly associated with an increase in protein content under delayed sowing condition. Storage protein Gluten was found within the range from 21.6 to 35.2% under D1 while 27.8 to 40.8% under elevated temperatures showed that gluten accumulation was also found increased under heat stress conditions **(Singh *et al*., 2021).**

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

The present study entitled "Morpho- physiological and biochemical characterization of wheat varieties (*Triticum aestivum* L.) sown at differential time intervals" was carried out at the Dr. N. E. Borlaug Crop Research Center, (E3 and E4 block), Govind ballabh Pant University of Agriculture and Technology, Pantnagar during two consecutive years of Rabi season 2018-19 and 2019-20. In this study the effect of two sowing time intervals (**D1;** 20th Nov 2018 and 22th November 2019) and (**D2;** 25th December, 2018 and 28th December, 2019) over 8 wheat varieties namely; UP2628, HD3086, UP2967, UP2784 (recommended for timely sowing), UP2526, UP2565, UP2748 and HD3059 (recommended for late sowing) were investigated. In comparison to D1 trial, D2 showed an elevation of (3.75ºC) at the time of tillering, (1.89 ºC) at heading, (3.75 ºC) at anthesis and (2.91 ºC) at the time of grain which was calculating by the average temperature of two years trial 2018-19 and 2019-20. The wheat varieties were evaluated for different morphological, physiological, biochemical parameters along with their enzyme activity and seed quality under both sowing conditions. The SDS PAGE analysis was also done to see the effect of elevated temperatures on seed storage proteins. The Salient findings of this investigation in response to elevated temperatures are summarized below:

1) Morphological parameters

- Plant height, tiller number, productive tillers were significantly reduced in all wheat varieties under D2 (sown in December month) as compared to D1 (sown in November month). A reduction of 1.71% to 1.69 % in plant height, 2.14% to 22.98 % in tiller number and 2.07% to 27.32% in productive tillers was observed in all wheat varieties under D2 as comparison to D1 sowing conditions. Least reduction in plant height and tiller number was observed in UP2565 and UP2526. While in productive tillers, least reduction was observed in UP2565.

- Flag leaf is also found highly sensitive towards elevated temperature, in all wheat varieties flag leaf length, width and area, fresh weight, dry weight and specific leaf weight was found reduced under D2 as compared to D1. A reduction of 3.83 % to 18.70 % in flag leaf length, 26.78% to 48.36 % in flag leaf width, 26.78% to 48.36 % in flag leaf area, 12.41% to 33.15 % in flag leaf fresh weight and 18.62 % to 25.99% in dry weight was observed in all wheat varieties sown in D2 as compared to D1. Least reduction in flag leaf length was observed in HD3086 while in width and area least reduction was reported in UP2628 due to elevation in temperatures. Similarly, least reduction in flag leaf fresh weight was observed in UP2748 and in dry weight was observed in UP2526.
- Leaf area index, specific leaf weight, total dry matter (at anthesis & at maturity) and internodal distance were significantly and negatively affected by difference in sowing timing. A reduction of 4.76% to 46.51 % in leaf area index, 0.45% to 24.39 % in specific leaf weight and 8.56 % to 39.50 % in total dry matter (at maturity) was observed in all wheat varieties under D2 as compared to D1. Least reduction in LAI and TDM (at maturity) due to elevated temperatures was observed in HD3059 while in specific leaf weight, least reduction was observed in UP2565 variety.
- 3rd internode was found most affected by elevated temperatures, a maximum reduction upto 16.81% was observed in 3rd internode as compared to 1st (up to 13.93%) and 2nd internodal distance (up to 9.54) under D2 as compared to D1.

2) Physiological parameters

- Values of relative water content, membrane stability index, chlorophyll fluorescence and canopy temperature were found significantly reduced in all wheat varieties under D2 as compared to D1.
- On comparing between two sensitive stage of wheat life cycle, relative water content, membrane stability index, chlorophyll fluorescence found higher during anthesis as compared to grain filling stage. Cooler canopy (higher

canopy temperature depression) was also observed during anthesis as compared to grain filling stage.

- At anthesis, a reduction of 16.73% to 48.05% in RWC, 36.31% to 66.22 % MSI, 5.65% to 20.19% in canopy temperature, 2.24% to 8.30% in CF was observed under D2 as compared to D1 in all wheat varieties. While during grain filling stage, a reduction of 16.73% to 48.05% in RWC, 36.31% to 66.22 % MSI, 5.65% to 20.19% in canopy temperature, 2.24% to 8.30% in CF was observed under D2 as compared to D1 sown wheat varieties. Least reduction in RWC and MSI due to elevated temperatures was observed in UP2967, for CTD in UP2748 and for CF, in UP2565.
- At the time of grain filling (15DAA), a reduction of 4.18% to 52.39% in RWC, 35.25% to 70.65% in MSI, 12.13% to 31.96% in CTD, 2.89% to 16.58% in CF was observed under D2 as compared to D1 conditions. Least reduction in RWC was observed for UP2967, in MSI it was observed for HD3086, in CTD, it was observed for UP2748 and in CF observed for UP2784.

3) Yield and Yield attributes

- Spike length, spike weight, total spike number and total spike weight, were found negatively and significantly affected by elevated temperatures in all wheat varieties under D2 as compared to D1.
- A reduction of 5.91% to 19.15% in spike length, 5.64 % to 37.44% in spike weight, 14.53% to 44.53% in spike number, 4.51% to 44.52% in total spike weight was observed in all wheat varieties under D2 as compared to D1. Least reduction in spike length was observed for UP2748 variety, in spike weight for UP2748 and HD3059, in spikes/plant for HD3086 and least reduction in total spike weight/plant was observed in UP2748 under D2 as compared to D1.
- Total spikelets per spike, grain number, 1000 grain weight, biological and grain yield were found negatively and significantly affected by elevated temperatures in all wheat varieties under D2 as compared to D1. A reduction

of 5.10% to 30.89% in spikelets/spike, 2.53% to 26.44% in grains/spike, 10.34% to 28.21% in 1000 grain weight, 6.89% to 23.11% in biological yield and 1.45% to 20.05% in grain yield was observed in all wheat varieties under D2 as compared to D1 conditions.

- Least reduction due to elevated temperatures in spikelets/spike was observed for UP2628 and HD3059, in grains/spike was observed for UP2967 and UP2526, in 1000 grain weight was observed for UP2748, in biological yield was observed for UP2526 while least reduction in grain yield due to elevated temperatures was recorded for UP2526 & UP2748.
- For wheat varieties UP2628 and UP2784, harvest index was found to be reduced due to elevated temperatures while in rest of varieties harvest index was significantly increased under D2 as compared to D1. Reduction in HI due to elevated temperatures was recorded in UP2628 and UP2784 variety (1.38 % and 12.40 % respectively) while a maximum increment in HI was observed in UP2967 (17.97 %) and minimum in UP2526 (2.08 %).
- The wheat varieties UP2628 (H.S.I= 0.90), UP2526 (H.S.I= 0.60), UP2565 (H.S.I= 0.70) and UP2748 (H.S.I=0.38) possess lower heat susceptibility index as compared to HD3086 (H.S.I= 1.29), UP2967 (H.S.I=1.32), UP2784 (H.S.I= 1.48) and HD3059 (H.S.I=1.09). Lowest H.S.I for grain yield was observed in UP2748 and highest H.S.I found in UP2784 variety.

4) Developmental Studies

- Days to heading, days to anthesis and days to physiological maturity were found significantly affected by elevated temperatures in all wheat varieties under D2 as compared to D1. Maximum reduction in number of days to heading due to elevated temperatures was recorded for HD3059 (10.32%) while least reduction was observed for UP2628 (4.86%). Maximum reduction in number of days to anthesis due to elevated temperatures was recorded in HD3059 (13.61%) while least reduction was observed for UP2565 (3.48%). Similarly, maximum reduction in number of days to physiological maturity due to elevated temperatures was recorded in UP2748 (20.00%) while least reduction was observed in HD3059 (12.25%).

5) Biochemical Parameters

- Chlorophyll pigments (total chlorophyll, chlorophyll a, chlorophyll b, chlorophyll a/b ratio), carotenoid content and NR activity was found significantly reduced due to elevated temperatures in all wheat varieties under D2 as compared to D1, while proline content was found significantly increased under D2 as compared to D1.
- On comparing between sensitive growth stages, chlorophyll pigments, carotenoids and NR activity were found higher during anthesis and tends to reduce as plants reaches towards grain filling while proline content was found increased in grain filling stage as compared to anthesis under D2 as compared to D1.
- At the of time anthesis, a reduction of 3.97 % to 31.38% in total chlorophyll, 6.07% to 30.06% in chlorophyll a, 8.54 % to 34.61 % in chlorophyll b, 2.56 % to 14.40% in chlorophyll a/b ratio and 10.24% to 75.75% in NR activity was observed under D2 wheat varieties as compared to D1 wheat varieties. However, an increment of 1.80 % to 65.56 % in proline content was observed under D2 as compared to D1 conditions
- least reduction in total chlorophyll content at the time of anthesis, under elevated temperatures was observed for UP2748 variety, in chlorophyll ‘a’ for UP2565, in chlorophyll ‘b’ for HD3086 and UP2748, in chl a/b ratio for UP2748, in carotenoid content for UP2565, in NR activity in UP2526. While, maximum increment in proline content due to elevated temperatures was recorded in UP2784.
- At the time of grain filling, a reduction of 3.98 % to 40.88 % in total chlorophyll, 1.82 % to 33.33 % in chlorophyll a, 5.36 % to 27.32 % in chlorophyll b, 0.60 % to 13.29 % in chlorophyll a/b ratio, 4.48 % to 51.96

% in carotenoids, 10.62 % to 75.75 % in NR activity was observed under D2 wheat varieties as compared to D1 sown wheat varieties. However, an increment of 3.79% to 75.05 % in proline content was observed under D2 as compared to D1.

- Least reduction in total chlorophyll content, chlorophyll b, chlorophyll a/b ratio, carotenoids, NR activity at the time of grain filling, under elevated temperatures was observed for UP2748 variety and in chlorophyll ‘a’ for UP2565. While, maximum increment in proline content due to elevated temperatures was recorded in UP2565.

Lipid peroxidation and antioxidant activity

- MDA content, was found to be significantly increased under D2 as compared to D1. On comparing between the two stages, MDA content in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage.
- An increment of 11.54 % to 79.66 % in MDA content was observed at the time of anthesis while 8.43 % to 69.66 % at the tine of grain filling was observed under D2 as compared to D1. At anthesis, least increment in MDA content due to D2 (elevated temperatures) was observed in HD3059. while at the time of grain filling, was observed in UP2748.
- Wheat varieties (recommended for late sowing) showed less increment in MDA content under elevated temperatures as compared to the wheat varieties (recommended for timely sown conditions).
- SOD and CAT activity was found to be significantly increased under D2 as compared to D1. On comparing between the two stages, SOD activity and CAT activity in all wheat varieties was found lower at the time of anthesis and tends to increase as varieties moves towards grain filling stage.
- At anthesis, an increment of 7.32% to 150 % in SOD activity and 2.86 % to 340% in CAT activity due to elevation in temperatures was observed under D2 wheat varieties while at grain filling an increment of 2.13% to 72.73 % in

SOD activity and 1.31 % to 108.33 % in CAT activity was observed under D2 as compared to D1 sown wheat varieties.

- At anthesis, maximum increment in SOD and CAT activity due to elevation in temperatures was recorded in UP2526 while at grain filling maximum increment in SOD due to elevation in temperatures was recorded in UP2526 and in CAT activity was recorded in UP2748.
- Wheat varieties (recommended for late sowing) showed more increment in antioxidant activity under elevated temperatures as compared to the wheat varieties recommended for timely sown conditions.

Seed quality of wheat

- Total soluble carbohydrates, starch content and amylopectin were found to be significantly, reduced under D2 as compared to D1, While, amylose content, grain nitrogen, total protein and storage proteins were found significantly increased.
- A reduction of 2.64 % to 24.36 % in total soluble carbohydrates, 2.64 % to 24.36 % in starch content, 0.57% to 5.03 % in amylopectin was observed under as compared to D1. While, an increment of 6.23 % to 99.42 % in amylose content, 26.23 % to 86.79 % in grain nitrogen, 19.11 % to 45.42 % in total proteins was observed in wheat varieties sown under D2 as compared to D1.
- Least reduction in total soluble carbohydrates and starch content was observed in UP2628, for amylopectin in UP2565, while maximum increase in grain nitrogen and total proteins was observed UP2565 and UP2748, for amylose content in UP2967 under elevated temperatures as compared to non-stress conditions.
- On comparing between the two selected wheat varieties, for SDS PAGE analysis, higher storage protein accumulation was observed in heat tolerant wheat variety (UP2748) as compared to heat sensitive variety (UP2784). According to quantitative analysis an increment of 334.62 % in albumin, 48.94% in globulin, 18.30 % in gliadin and 28.33 % in gluten was found in

variety UP2784 under D2 as compared to D1. While variety UP2748 showed 152.42 % increment in albumin, 16.19 % in globulin, 113.12 % in gliadin and 104.60 % in gluten under D2 as compared to D1.

- Number of bands for all wheat storage proteins were found increased under D1 as compared to D2 while banding patterns was found darker for heat tolerant variety as compared to heat sensitive variety. In total proteins, 14.30 KDa, 43.00 KDa, 20.00 KDa, 20.00 KDa to 29.00 KDa, 14.30 KDa and 43.00 KDa molecular weight protein were differentially expressed under both sowing conditions. In albumins, 14.30 KDa, 20.00 KDa, 29.00 KDa, 66.00KDa and 97.40 KDa molecular weight protein were found significantly increased which means various heat shock proteins, responsible for more accumulation of storage protein albumin under stress condition. In globulin, no big difference in banding patterns in both varieties under both sowing conditions was observed. Also in gliadin, not much difference was observed in banding patterns of wheat varieties under D2 in comparison to D1. Overall, much faded and light bands observed for heat-sensitive wheat variety under D2 as compared to D1 conditions. Maximum number of bands was observed for gluten protein as this could be due to maximum increment in gluten protein under elevated temperature as compared to others. More number of bands (darker) present in both wheat varieties (heat sensitive and tolerant) under D2 condition as compared to D1 conditions as gluten positively affected by elevated temperatures.

6) Correlational Analysis

Correlational analysis between morphological parameters showed that due to the delay in sowing (D2), the relationship between grain yield with other morphological traits were significantly affected, but no significant difference was found between physiological parameters under both sowing months. Due to delay in sowing, plant experiences heat stress, as a result, the relation between the antioxidant defense system and osmolytes were significantly and positively increased under D2 as compared to D1. A significant and negative correlation between yield and yield parameters was also observed under D2 wheat varieties as compared to D1.

Conclusion

A difference of 30 day time interval (between two sowing months Nov& Dec) causes elevation in air temperatures as 3.75 ºC at anthesis and 2.91 ºC at grain filling, two sensitive growth stages of wheat life cycle. On the basis of the whole study, wheat varieties recommended for timely sown conditions (UP2628, HD3086, UP2967, UP2784) were found significantly affected by elevated temperatures as compared to wheat varieties recommended for late sowing conditions (UP2526, UP2565, UP2748 and HD3086). Least reduction in most of the morphological parameters, yield attributes with maximum increment in antioxidant activities, grain quality parameters were observed in varieties UP2748 and UP2565 under elevated temperatures as compared to non-stress conditions, showed the inherit capability of both the varieties performing well under both sowing conditions (November and December). However, out of wheat varieties recommended for timely sown conditions (HD3086 with H.S.I =1.29, UP2967 with H.S.I = 1.32, UP2784, with H.SI = 1,48), UP2628 with lower H.S.I (0.90) and maximum grain yield, biological yield, membrane stability index (during both sensitive stage) even under elevated temperatures were found moderately tolerant towards stress conditions and can be recommended for December sowing in tarai region. By using such multiparametric approach, it is easier to predict the inherit characteristic behavior of a variety in a particular area. More research work should be continued for screening and selecting the cultivars which are based on their morpho-physiological and biochemical traits as they are stable, easy to measure, heritable and strongly correlated with the yield.

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Appendices

Appendix I

Percent (%) reduction in mean values of morphological parameters of different varieties under D2 as compared to D1.

Varieties	Plant Height	Tiller No.	Prod. Tillers	Flag leaf Length	Flag leaf Width	Flag leaf Area	Flag leaf Fresh weight	Flag leaf Dry weight
UP2628	16.25	16.08	14.15	15.73	3.68	26.78	30.67	21.83
HD3086	14.81	11.81	12.51	3.83	44.22	37.05	16.35	21.25
UP2967	5.92	22.95	13.98	16.84	25.71	38.30	33.15	26.32
UP2784	8.92	22.98	27.32	14.84	39.50	48.36	22.20	25.99
UP2526	1.71	2.59	4.32	7.69	38.55	43.28	27.50	18.62
UP2565	1.69	2.14	2.07	18.70	31.47	38.75	15.05	24.73
UP2748	7.80	7.58	8.62	8.98	37.86	37.68	12.41	24.53
HD3059	3.43	7.22	5.20	7.09	28.00	32.96	20.01	24.56

Varieties	SLW	LAI	TDM (at anthesis)	TDM (at maturity)	1st IND	2nd IND	3rd IND
UP2628	17.19	22.38	14.54	19.81	13.93	9.07	16.81
HD3086	24.39	46.51	15.72	17.22	10.56	9.12	14.48
UP2967	8.36	40.62	2.05	20.65	12.00	8.90	14.01
UP2784	14.29	14.49	20.49	39.50	6.57	9.54	12.29
UP2526	2.79	12.38	7.20	11.35	6.06	7.95	10.18
UP2565	0.45	18.85	1.24	13.93	6.30	6.63	9.85
UP2748	5.99	18.12	2.59	13.84	5.49	7.07	9.05
HD3059	2.80	4.76	6.61	8.56	8.07	7.76	7.65

Note; SLW; Specific leaf weight, LAI: Leaf Area Index, TDM; Total Dry Matter, IND; Inter nodal Distance

Appendix II

Percent (%) reduction in mean values of physiological parameters of different varieties under D2 as compared to D1.

Varieties	RWC		MSI		CTD		CF	
	At anthesis	**15DAA**	**At anthesis**	**15DAA**	**At anthesis**	**15DAA**	**At anthesis**	**15DAA**
UP2628	29.57	52.39	46.02	50.46	9.14	13.65	3.27	14.14
HD3086	29.24	26.28	53.30	35.25	11.20	31.96	3.25	10.29
UP2967	16.73	4.18	36.31	52.06	20.19	20.01	8.30	12.32
UP2784	32.41	13.35	64.16	63.99	16.21	28.10	7.36	2.89
UP2526	48.05	41.05	64.13	56.81	7.97	21.29	2.24	3.59
UP2565	25.36	18.06	64.57	70.65	10.70	24.19	2.66	7.33
UP2748	33.44	13.57	66.22	58.59	5.65	12.13	2.58	9.81
HD3059	20.62	15.43	62.05	63.49	7.14	31.57	2.87	16.58

Note; RWC; Relative water content, MSI: Membrane stability Index, CTD; Canopy Temperature Depression, CF; Chlorophyll fluorescence.

Appendix III

Percent (%) reduction in mean values of yield and yield attributes of different varieties under D2 as compared to D1.

Varieties	Spike length	Spike weight	Total spikes number/plant	Total spikes weight/plant
UP2628	15.63	24.33	18.35	9.26
HD3086	14.26	32.76	14.53	20.65
UP2967	15.86	21.38	44.53	37.05
UP2784	13.07	37.44	30.95	33.69
UP2526	13.34	23.27	29.78	44.52
UP2565	10.00	22.68	41.73	33.40
UP2748	5.91	8.66	19.99	4.51
HD3059	19.15	5.64	20.69	33.73

Varieties	Total spikelets/spike	Grains/ spike	1000 grains weight	Biological yield	Grain yield	Harvest Index
UP2628	5.10	26.44	17.55	9.48	7.89	1.4
HD3086	30.89	16.04	20.30	23.11	16.09	-9.3 (Inc)
UP2967	24.15	2.53	22.60	19.11	16.72	-18.0 (Inc)
UP2784	8.56	6.50	28.21	19.43	20.05	12.4
UP2526	18.26	3.78	22.54	3.44	1.45	-2.1(Inc)
UP2565	29.03	22.86	17.39	19.10	3.77	-16.1 (Inc)
UP2748	17.00	8.18	10.34	6.86	2.93	-8.8 (Inc)
HD3059	6.16	19.79	15.88	20.85	11.87	-10.5 (Inc)

Appendix IVa

Percent (%) reduction in mean values of number of days taken to heading, anthesis and physiological maturity from date of sowing of different wheat varieties under D2 as compared to D1.

Varieties	Days to Heading	Days to Anthesis	Days to Physiological maturity
UP2628	4.86	5.23	18.42
HD3086	8.20	8.72	15.53
UP2967	8.27	10.34	16.29
UP2784	9.23	9.30	19.63
UP2526	6.64	8.53	17.04
UP2565	8.70	3.48	15.23
UP2748	8.20	10.85	20.00
HD3059	10.32	13.61	12.25

Appendices IVb

Percent reduction in biochemical parameters (except in proline; % increment) of different wheat varieties under D2 in comparison to D1.

	Total Chlorophyll		Chlorophyll a		Chlorophyll b		Chlorophyll a/b ratio	
Varieties	At anthesis	15DAA	At anthesis	15DAA	At anthesis	15DAA	At anthesis	15DAA
UP2628	31.38	40.88	17.27	33.33	26.15	21.88	13.80	11.96
HD3086	8.78	19.60	7.34	21.22	8.54	15.89	6.19	5.83
UP2967	12.22	24.83	7.33	18.39	34.61	27.32	8.77	7.06
UP2784	12.01	9.70	30.06	2.65	17.73	9.97	14.40	13.29
UP2526	5.47	10.01	9.25	1.82	22.63	6.52	5.38	4.18
UP2565	6.32	5.08	6.07	4.53	12.36	10.57	7.72	2.66
UP2748	3.97	3.98	9.73	3.96	9.77	5.36	2.56	0.60
HD3059	4.62	12.90	7.46	18.18	14.21	9.24	6.34	11.14

	Carotenoid Content		Proline Content (Inc)		NR Activity	
Varieties	At anthesis	15DAA	At anthesis	15DAA	At anthesis	15DAA
UP2628	49.08	51.96	63.57	56.36	26.73	23.22
HD3086	8.08	14.23	1.80	3.79	24.46	46.32
UP2967	8.60	17.24	14.32	37.82	21.98	6.49
UP2784	16.35	8.81	65.56	59.80	31.31	75.75
UP2526	9.04	9.63	41.77	16.45	10.24	67.36
UP2565	4.70	7.75	38.05	75.05	27.96	38.10
UP2748	10.91	4.48	15.55	40.08	20.80	1.79
HD3059	13.56	13.83	27.79	43.70	17.81	42.46

Appendix IVc

Percent increment in Lipid peroxidation and Antioxidants of different wheat varieties under D2 in comparison to D1.

	MDA Content		SOD Activity		CAT Activity	
Varieties	**At anthesis**	**15DAA**	**At anthesis**	**15DAA**	**At anthesis**	**15DAA**
UP2628	79.66	32.15	27.78	51.85	31.54	79.25
HD3086	45.49	36.28	7.32	2.13	3.17	29.41
UP2967	44.03	41.33	25.00	24.49	2.86	43.75
UP2784	39.83	69.62	10.00	5.71	46.67	1.31
UP2526	41.99	38.05	150.00	72.73	105.66	15.71
UP2565	33.60	8.57	11.11	5.00	340.00	37.53
UP2748	12.09	8.43	25.58	10.34	28.74	108.81
HD3059	11.54	16.60	78.26	22.22	65.06	94.97

	Specific Enzyme Activity (SOD)		Specific Enzyme Activity (CAT)	
Varieties	**At anthesis**	**15DAA**	**At anthesis**	**15DAA**
UP2628	65.33	96.48	1.66	38.53
HD3086	42.84	35.94	19.31	5.06
UP2967	44.63	44.04	7.20	24.24
UP2784	32.44	27.28	21.82	33.33
UP2526	197.77	105.73	72.67	4.36
UP2565	57.40	48.74	210.61	2.36
UP2748	82.62	60.47	11.01	43.59
HD3059	147.33	69.58	18.97	40.52

Note; MDA ; Malonaldehyde Assay, SOD: Superoxide Dismutase Activity , CAT; Catalase Activity

Appendix IVd

Percent reduction in grain quality (except in amylose content, amylose to amylopectin ratio, grain nitrogen and protein content; % increment) of different wheat varieties under D2 in comparison to D1.

Varieties	Total soluble carbohydrates	Starch content	Amylopectin
UP2628	24.37	24.20	2.82
HD3086	22.44	21.44	9.94
UP2967	19.79	18.79	9.48
UP2784	13.90	11.96	1.94
UP2526	5.80	5.80	3.64
UP2565	14.06	14.06	0.57
UP2748	6.67	6.67	3.52
HD3059	2.65	2.65	5.04

Varieties	Amylose content (Inc.)	Ratio of Amylose to Amylopectin (Inc.)	Grain nitrogen (Inc.)	Total soluble protein (Inc.)
UP2628	34.30	2.82	26.23	29.39
HD3086	72.73	9.94	57.92	33.10
UP2967	99.42	9.48	35.32	15.70
UP2784	19.24	1.94	63.28	20.40
UP2526	47.35	3.64	56.75	19.11
UP2565	6.23	0.57	86.79	41.66
UP2748	36.19	3.52	53.57	45.42
HD3059	66.34	5.04	50.29	38.75

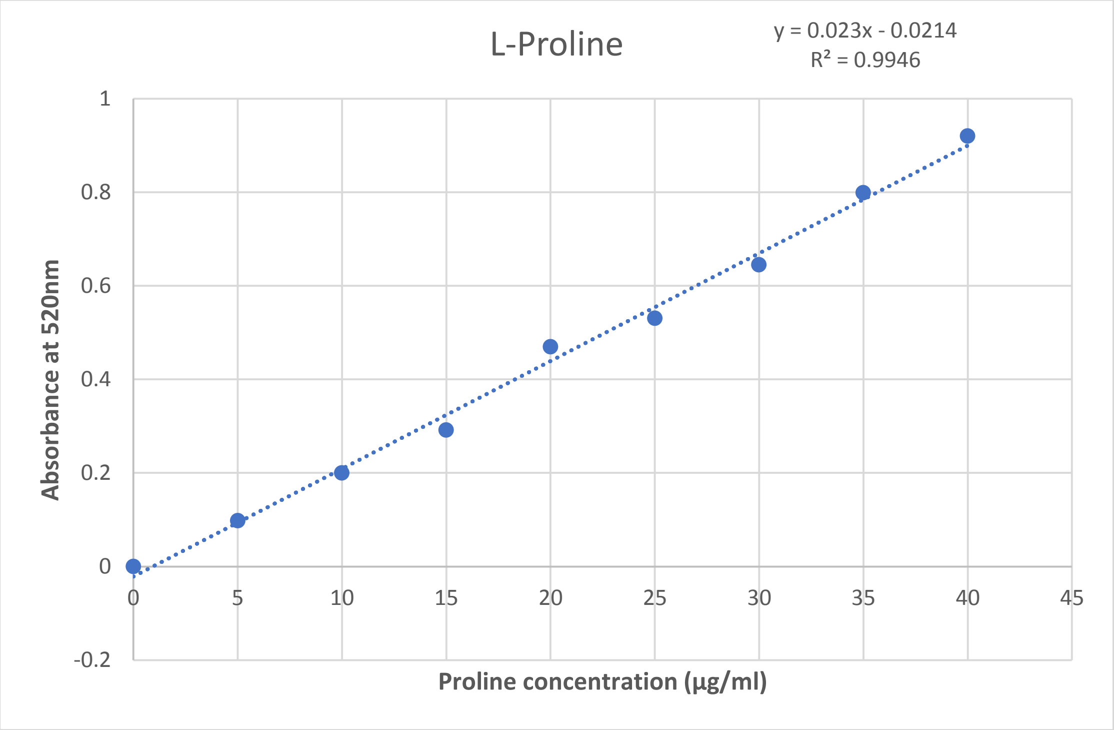
Appendix V

Percent increment in storage proteins (in grains) of selected wheat varieties under D2 in comparison to D1.

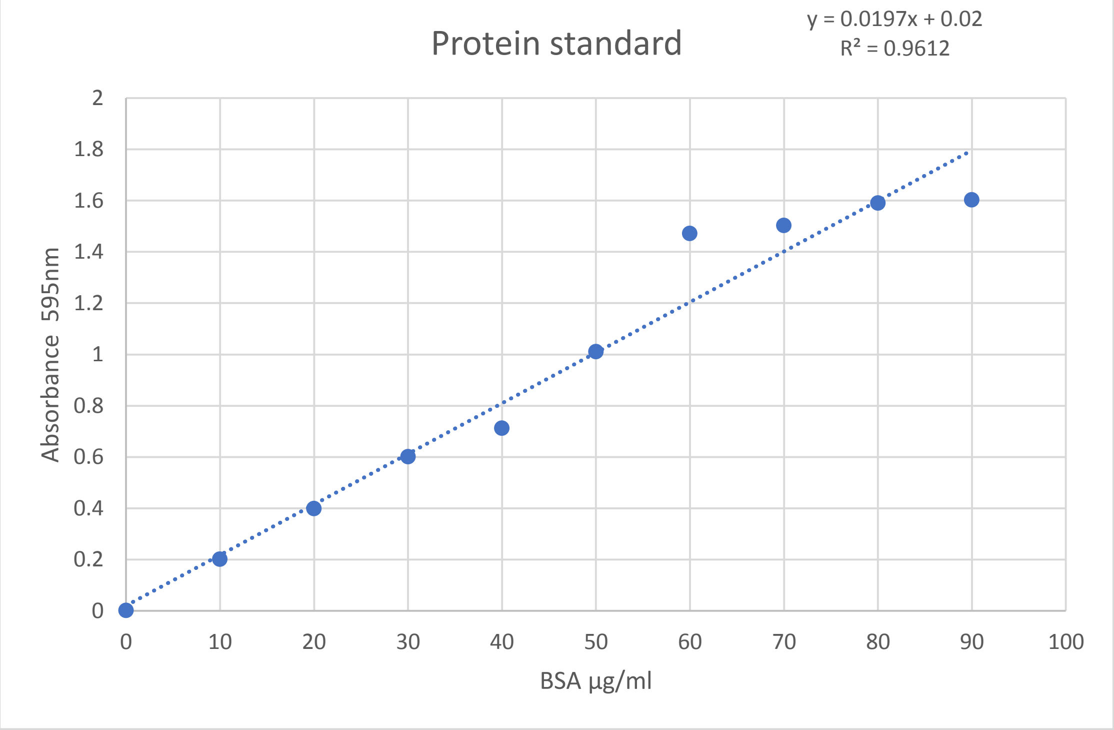
	Albumin	Globulin	Gliadian	Gluten
UP2784	334.62	48.94	18.30	28.33
UP2848	152.42	16.19	113.12	104.60

Appendix VI

Standard graph for Proline

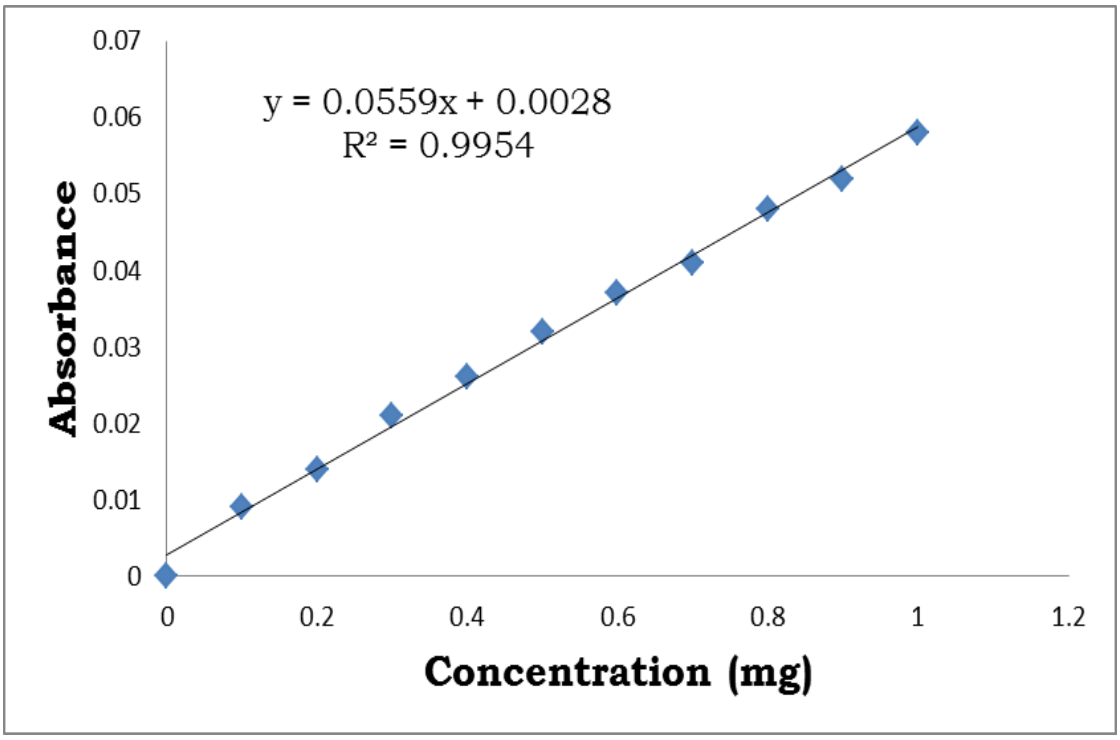


Standard graph for Protein



Standard graph for Carbohydrates and Starch

Standard graph for Amylose



Appendix VII

Weather report of two years Data 2018-19 and 2019-20. (IN ANOTHER FOLDER)
Station name Pantnagar

Latitude 29 deg. N Longitude 79 deg.30’E Altitude 243.84 m. AMSL

Weather Data November to May, 2018-2019

Month	Date	Year	Temperature ^{0}C		R. Humidity %		Rainfall (mm)	Wind Velocity (Km/hr)	Sun shine hrs.	Evap. (mm)
			Max.	Min.	0712 am	1412 pm				
Nov	1-7	2018	29.01	13.05	88.14	55.14	4.2	3.24	6.54	2.74
	8-14	2018	26.97	12.44	92.29	58.14	0	2.36	6.94	1.9
	15-21	2018	26.3	10.83	93.14	60.71	0	1.93	7.17	2.51
	22-28	2018	26.3	10.77	94.14	53	0	1.81	8.11	2.6
	29-30	2018	25.75	10.15	94.5	61.5	0	2.15	5.3	2.4
Dec	1-7	2018	24.36	8.63	92.86	61.86	0	1.86	5.3	2.04
	8-14	2018	22.81	7.01	93.71	61.14	0.8	2.04	6.39	1.96
	15-21	2018	23	5.04	96.14	52.28	0	1.81	7.41	2.04
	22-28	2018	20.77	3.84	98.29	49.71	0	1.98	6.3	1.46
	29-31	2018	19.5	4.77	95.33	56	0	-	6.87	1.3
Jan	1-7	2019	21.3	6.04	91	60.29	0	-	6.01	1.64
	8-14	2019	21.73	5.7	94.43	57	0	-	5.76	1.7
	15-21	2019	21.73	5.66	93	53	0	2.13	6.06	1.83
	22-28	2019	20.53	8.57	88.43	56.57	13.4	3.79	3.76	2.1
	29-31	2019	20.9	6.33	94.33	59.66	0	1.3	7.3	1.6
Feb	1-7	2019	21.36	8.56	93.43	67.86	0.14	1.87	4.63	1.81
	8-14	2019	22	9.13	93	61.29	2.33	3.18	5.53	2.04
	15-21	2019	22.46	11.7	93.14	66.14	2.17	3.07	4.07	2.06
	22-28	2019	23.88	9.86	92	63.71	0.46	3.01	5.06	2.57
Mar	1-7	2019	22.79	9.04	90.71	64.86	3.6	2.09	7.53	2.76
	8-14	2019	26.89	10.47	85.71	45	0	1.59	7.43	3.77
	9-21	2019	28.99	11.76	86.57	45.71	2.6	2.04	8.27	3.43
	22-28	2019	30.83	12.48	88	46.57	0	0.9	7.73	5
	29-31	2019	33.33	16.6	83	40	0	1.26	8.67	4.6
Apr	1-7	2019	33.76	17.5	72.71	42.86	0	4.11	8.58	5.73
	8-14	2019	34.71	17.74	73.43	38.43	3.2	4.6	8.71	6.69
	15-21	2019	33.5	17.67	73.29	34.29	9.2	5.49	7.77	6.13
	22-28	2019	37.93	20.39	63.86	29	0	5.39	10.04	8.63
	29-30	2019	39.75	16.9	73.5	14	0	5.15	11.05	10.35
May	1-7	2019	39.1	20.91	84.86	17.29	0	7.56	9.94	10.23
	8-14	2019	40.66	20.6	51.86	21	0	7.63	9.84	10.47
	15-21	2019	**32.14**	**20.74**	**64.14**	**24.57**	**0**	**7.05**	**7.4**	**8.9**
	22-28	2019	30.67	21.06	56.71	16.71	**0**	7.28	9.98	9.29
	29-31	2019	41.33	21.6	45.33	23.33	**0**	6.93	10.9	11.83

Weather Data November to May 2019-2020

Month	**Date**	**Year**	**Temperature ^{0}C**		**R. Humidity %**		**Rainfall (mm)**	**Wind Velocity (Km/hr)**	**Sun shine hrs.**	**Evap. (mm)**
			Max.	**Min.**	**0712 am**	**1412 pm**				
Nov	1-7	2019	29.23	15.76	87.71	57.43	0	3	2.91	2.07
	8-14	2019	29	13.36	88.28	39.57	0	2.73	17.57	2.56
	15-21	2019	28.08	12.71	92.57	42.28	0	2.8	6.17	2.16
	22-28	2019	26.43	12.64	91.43	48.86	0	2.08	4.84	2.04
	29-30	2019	24.75	10.65	92.5	51.5	29.2	3.9	5.86	3.75
Dec	1-7	2019	23.38	8.47	94.57	51.43	0	1.98	6.94	1.94
	8-14	2019	22.07	9.66	94.10	55.14	39.9	4.48	5.53	3.21
	15-21	2019	16.01	9.1	94.86	74.43	0	4.07	2.6	1.27
	22-28	2019	14.7	6.33	95.57	78.71	0	4.57	3.14	1.47
	29-31	2019	11.06	6.16	98	72.33	0	3.46	0.23	0.9
Jan	1-7	2020	20.13	7.51	92	61.28	1.63	2.06	5.03	2
	8-14	2020	14.4	8.28	95.43	81.14	4.03	2.23	1.2	1.43
	9-21	2020	18.07	9.9	94.43	78.71	6.87	1.16	3.34	1.7
	22-28	2020	17.73	6.78	92.71	70.71	0.1	1.8	4.2	1.67
	29-31	2020	17.76	7.73	94	71	9.93	3.96	4.2	3.23
Feb	1-7	2020	19.03	4.46	96.43	52.71	0	2.26	6.91	2.13
	8-14	2020	21.64	5.83	95.28	53.57	0	1.2	8.38	2.74
	9-21	2020	23.97	9.71	94.57	60.43	1.88	3.27	6.73	3.16
	22-28	2020	23.46	11.88	91.71	59.28	1.66	1.44	4.41	2.53
	29	2020	24.5	11.4	98	54	0	1.1	7.1	2.5
Mar	1-7	2020	23.71	12.24	89.28	52.43	4.21	2.9	7.2	3.68
	8-14	2020	25.11	13.17	86.57	54	2.72	4.07	7.76	3.26
	9-21	2020	26.83	11.57	90.14	44.57	0.28	1.86	9.07	3.63
	22-28	2020	27.97	15.77	83.28	42.71	0.14	3.07	7.57	4.06
	29-31	2020	30.06	12.93	90.33	32.66	0	5.03	10.53	5.23
Apr	1-7	2020	32.4	13.06	79.86	26.43	0	2.54	10.24	6.21
	8-14	2020	34.94	15.87	71	24.57	0	2.33	.67	6.57
	9-21	2020	35.37	20.11	57.71	29.86	0	2.63	5.97	6.78
	22-28	2020	32.01	18.73	63.86	44.43	10.11	3.93	7.24	5.45
	29-30	2020	30.35	21.45	68.5	54	0	2.3	8.8	5.65
May	1-7	2020	32.16	19.74	73.57	44.57	4.24	4.23	8.9	5.47
	8-14	2020	32.54	20.43	70.71	49.14	4.08	2.51	8.78	6.07
	9-21	2020	**37.43**	**18.53**	**62.71**	**25.14**	**0**	**3.74**	**7.78**	**7.9**
	22-28	2020	39.24	23.54	72.42	28.71	**0**	2.91	11.11	9.01
	29-31	2020	32.7	22.56	75.66	69.66	**0.93**	7.16	4.96	5.86
June	1-7	2020	31.43	22.43	80.14	52.43	**9.63**	2.14	6.01	5.33
	8-14	2020	36	26.23	72	51.43	**0.4**	2.33	8.47	6.43
	9-21	2020	34.07	25.77	79.43	62.57	**10.2**	2.21	7.74	6.48
	22-28	2020	32.93	25.96	88.28	68.28	**9.34**	0.9	5.14	5.57
	29-30	2020	31.5	26.15	83.5	70	**7.7**	5.8	0.35	6.1

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3.	**B.Sc. (Z.B.C,)**	D.S.B. Campus, Kumaun University, Nainital	2015	**64.45**
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  - **Papers published from thesis : (1)** "Impact of elevated temperatures on water status and membrane integrity and antioxidant activity in flag leaves of wheat varieties (*Triticum aestivium*) sown at differential time intervals
  - **Papers published other than thesis: 0**
- **Conferences/Seminars/Workshops/Trainings Attended: 4**
- **List of papers presented in conference/seminar during degree programme: 4**
  - ✓ Chandra, T., Jaiswal, J. P., Shankhdhar, S. C. and Shankhdhar, D. (2019). Morphological and biochemical characterization of wheat (*Triticum aestivium* L.) varieties under different sowing dates; National Conference of Plant Physiology (NCPP), Thrissur, Kerala, 19-21 Dec., pp.97.
  - ✓ Elucidation of physiological and biochemical markers of rice genotypes under different nitrogen levels; 4th International Plant Physiology Congress, CSIR- National Botanical Research Institute, Lucknow, India, 2-5 Dec., pp.314.
  - ✓ Biochemical characterization of rice genotypes under different nitrogen levels; Uttarakhand State Council for Science and Technology, Dehradun India, 26-28 Feb., pp.26.
  - ✓ Characterizing the physiological traits for improving heat tolerance in wheat (*Triticum aestivum* L.) under different sowing conditions; 3rd Plant Science Research Meet (PRSM), Dehradun, Uttarakhand, 26-27 Sep., pp.68.

- **Software skills**: STPR 15, SPTPR 2, OPSTATS
- **Professional skills**: Nil
- **Professional Affiliations:** Part time Graduate teaching assistant during II sem 2015-16 and 2016-17.
- **Awards/Honours/Achievements:** Nil

Tanvi Chandra.

Place : Pantnagar

Date : September, 2021

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Sem. & year of admission	: 1st, 2017-18	**Degree**	: Ph.D.
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Minor	: Molecular Biology and Biotechnology		
Topic	: **“Morpho-physiological and biochemical characterization of wheat varieties (*Triticum aestivum* L.) sown at differential time intervals”**		
No. of pages	: 229	**Advisor**	: Dr. Deepti Shankhdhar

ABSTRACT

Wheat (*Triticum aestivum* L.*),* a major staple food resource for the world’s population. In present scenario climate changes and elevated temperatures are the big issues that limit its production. An inappropriate timing or delay in sowing is also responsible for sudden elevation in air temperatures during sensitive growth stages of wheat life cycle. Being a winter crop, variability in climates is one of the biggest environmental threats to Indian wheat. The present investigation was carried out to evaluate the effect of elevated temperatures due to variation in sowing timings (November and December) on morpho-physiological and biochemical parameters of 8 varieties of wheat namely; UP2628, HD3086, UP2967, UP2784, UP2526, UP2565, UP2748 and HD3059, conducted during the two consecutive years of Rabi season 2018-19 and 2019-20 in Pantnagar (tarai region). A delay of 30 days in sowing, results elevation in air temperatures; 3.7ºC at tillering, 1.9ºC at heading, 3.7ºC at anthesis and 2.9ºC at grain filling. The morphological, physiological and biochemical parameters, yield and yield related attributes, days taken to complete developmental stages were significantly reduced under December sown wheat varieties as compared to November sown wheat varieties. 3rd internodal distance was found affected by elevated temperatures as compared to 1st and 2nd. On comparing between sensitive growth stages; thermo tolerant traits such as relative water content, membrane stability index, chlorophyll fluorescence, canopy temperatures, chlorophyll pigments and NR activity was significantly decreased during grain filling stage as compared to anthesis. While antioxidants and proline content were found increased as the varieties moves towards grain filling. Quality parameters, carbohydrates, starch and amylopectin were found negatively affected by elevated temperatures while grain nitrogen, storage proteins, amylose content, amylose to amylopectin ratio was positively influenced in December sown wheat varieties. Positive and significant correlation between grain yield and other parameters was found in wheat varieties sown in November while under December sown, relations between the grain yield and other parameters were significantly changed except the correlations among physiological traits, in which non-significant difference was observed between two sowing months.

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Advisor

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प्रमुख विषय : पादप कार्यिकी विभाग : पादप कार्यिकी
लघु विषय : आण्विक जीवविज्ञान और जैव प्रौद्योगिकी
विषय : "अंतर समय अंतराल पर बोई गई गेहूं की किस्मों (ट्रिटिकम एस्टिवम एल.) का रूपात्मक- कार्यिकी और जैव रासायनिक लक्षण वर्णन"
पृष्ठ संख्या : 229 सलाहकार : डॉ दीप्ति शंखधार

सारांश

गेहूँ (ट्रिटिकम एस्टिवम एल.), विश्व की जनसंख्या के लिए एक प्रमुख प्रधान खाद्य संसाधन है। वर्तमान परिदृश्य में जलवायु परिवर्तन और तापमान मे बड़त बड़े मुद्दे हैं जो इसके उत्पादन को सीमित करते हैं। गेहूं के जीवन चक्र के संवेदनशील विकास चरणों के दौरान हवा के तापमान में अचानक वृद्धि के लिए अनुचित समय या बुवाई में देरी भी जिम्मेदार है। सर्दियों की फसल होने के कारण, जलवायु में परिवर्तनशीलता भारतीय गेहूं के लिए सबसे बड़े पर्यावरणीय खतरों में से एक है। वर्तमान जांच, गेहूं की 8 किस्मों के रूपात्मक- कार्यिकी और जैव रासायनिक मानकों पर बुवाई के समय (नवंबर और दिसंबर) में भिन्नता के कारण ऊंचे तापमान के प्रभाव का मूल्यांकन करने के लिए की गई थी; गेहूं की 8 किस्मों: UP2628, HD3086, UP2967, UP2784, UP2526, UP2565, UP2748 और HD3059 को पंतनगर (तराई क्षेत्र) मे लगातार वर्ष 2018-19 और 2019-20 के रबी के मौसम मे दौरान आयोजित किया गया। बुवाई में 30 दिनों की देरी के कारण हवा के तापमान में निमनानसार वृद्धि पाई गई ; जुताई पर 3.7 °C, शीर्ष पर 1.9 °C, पुष्पण पर 3.7 °C और अनाज भरने पर 2.9 °C नवंबर में बोई गई गेहूं की किस्मों की तुलना में दिसंबर में बोई गई गेहूं की किस्मों के तहत रूपात्मक, - कार्यिकी और जैव रासायनिक मापदंडों, उपज और उपज संबंधी विशेषताओं, विकास के चरणों को पूरा करने में लगने वाले दिनों में काफी कमी आई। तीसरी इंटरनोडल दूरी पहले और दूसरे की तुलना में ऊंचे तापमान से प्रभावित पाई गई। संवेदनशील विकास चरणों के बीच तुलना करने पर; पुष्पण की तुलना में अनाज भरने के चरण के दौरान तापरोधक लक्षण जैसे सापेक्ष जल सामग्री, झिल्ली स्थिरता सूचकांक, क्लोरोफिल प्रतिदीप्ति, चंदवा तापमान, क्लोरोफिल वर्णक और एनआर गतिविधि में काफी कमी आई थी जबकि एंटीऑक्सीडेंट और प्रोलीन सामग्री बढ़ी हुई पाई गई गुणवत्ता मानदंड जैसे कार्बोहाइड्रेट, स्टार्च और एमाइलोपेक्टिन ऊंचे तापमान से नकारात्मक रूप से प्रभावित पाए गए, जबकि अनाज मे नाइट्रोजन, भंडारण प्रोटीन, एमाइलोज सामग्री, एमाइलोज और एमाइलोपेक्टिन अनुपात सकारात्मक रूप से दिसंबर में बोई गई गेहूं की किस्मों में प्रभावित पाए गए नवंबर में बोई गई गेहूं की किस्मों में अनाज की उपज और अन्य मापदंडों के बीच सकारात्मक और महत्वपूर्ण सहसंबंध पाया गया, जबकि दिसंबर में बोई गई, अनाज की उपज और अन्य मापदंडों के बीच गैर-महत्वपूर्ण अंतर देखा गया हालकी कार्यिकी संबंधों को छोड़कर बाकी सहसंबंधों मे बदलाव देखा गया था

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