STARCH METABOLISM AND END USE QUALITY OF DURUM WHEAT IN RESPONSE TO HIGH

TEMPERATURE STRESS

By

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Dedicated

To

My Beloved Parents

CERTIFICATE-I

This is to certify that this thesis entitled, "**Starch metabolism and end use quality of durum wheat in response to high temperature stress"**, submitted for the degree of **Master of Science,** in the subject of **Plant Physiology** of the CCS Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Kavita Bansal (2005BS123M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that this thesis entitled, "**Starch metabolism and end use quality of durum wheat in response to high temperature stress"**, submitted by **Kavita Bansal (2005BS123M)** to the CCS Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of **Master of Science,** in the subject of **Plant Physiology**, has been approved by the Student's Advisory Committee after an oral examination on the same in collaboration with an external examiner.

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CHAPTER-I

Introduction

Among various constituents of food, cereals particularly wheat contributes more than 60% of the food calories and is an important agricultural crop. It is the cheapest source of carbohydrates and proteins and a staple food for about one third population of the world. Wheat, on the world scenario, covers 32% of total area under cereals. Major wheat producing countries are China, India, USA, Russia, Canada and Australia. Wheat is the second most important food crop in India.

Among different wheat species grown world wide, durum wheat (4x) is next of importance after bread wheat (6x). The production of durum wheat in world during 2005-06 was 26 million tons and down sharply from last year's record of around 33 million. Durum wheat is the second most important wheat species occupying nearly 11% of the total wheat area of India. With an area of nearly 2.0 million hectares, India could become one of the biggest producers and exporters of

durum wheat in the world (Nagarajan *et al.*, 1998). Haryana is ranked sixth in area under wheat in the country but is only next to Punjab in terms of its contribution to central pool (Yunus *et al.*, 1996).

Advantage due to which the durums can be the demanding commodity in future are value addition potential, better instant food products, resistant to Karnal Bunt disease, minimum quarantine issues, better price in the market, diversification or varietal mosaic, worldwide preferred products and suitable for marginal areas (Shoran *et al.*, 2002). Durum wheat contains 1.5 to 2.0% higher protein than bread wheat. In addition, it contains higher β–carotene (precursor of vitamin A) too, required to solve the problem of malnutrition among children and rural population (Madan *et al.*, 2006).

Howard (1924) stated that 'wheat production in India is a gamble in temperature', which remains valid even today. Continual heat stress can be a problem in 40% of the irrigated wheat sowing areas of the world (Fisher and Byerlee, 1991). High temperature in the month of March-April is not congenial for grain growth, hence, results into forced crop maturity. This results, not only in reduced number of grains spike^{-1} but also causes poor grain filling thus produces shriveled grains with low test weight (Shpiler and Blum, 1991; Nagarajan and Rane, 2002).

As temperature rises above 18-22^{0}C, the observed decrease in the duration of deposition of dry matter in the kernel is not accompanied by a compensating increase in the rate of grain filling with the result that grain weight and yield is diminished at high temperature (Bhullar and Jenner, 1986; Jenner, 1991; DuPont and Altenbach, 2003). High temperature affects the yield of crop by reducing various physiological parameters such as plant water status, crop growth, photosynthesis, cell membrane permeability (Dhanda and Munjal, 2006). Among the yield attributes affected severely by high temperature are number of shoots plants^{-1}, number of grains ear^{-1} and 1000-grain weight (Stone and Nicolas, 1995; Reynolds *et al.*, 1994).

High temperature affects final grain weight by reducing the duration of grain filling due to suppression of current photosynthesis and directly by inhibiting starch biosynthesis in the endosperm (Fokar *et al.*, 1998). High temperature influenced the rate and duration of protein accumulation and starch deposition (Altenbach *et al.*, 2003).

Reduced starch content accounts for most of the reduction in grain dry matter at high temperature. Soluble starch synthase, the key enzyme synthesizing starch in wheat kernel seems to be most sensitive to high temperature stress. A better understanding of the mechanisms involved in loss of activity of starch synthesizing enzymes of two

genotypes differing in their sensitivity to high temperature will open up new opportunities to breed temperature tolerant genotypes. The adverse climatic conditions encountered during grain filling can affect the biochemical composition of the grain which in turn affects the processing quality of the grain. To maintain consistent wheat quality varieties which can tolerate changes in climatic or cultivation conditions would be needed. Keeping this in view the present study was planned with the following objectives :

1. To find out the biochemical and physiological basis of high temperature induced changes in sucrose to starch conversion.
2. To determine the response of high temperature stress on grain quality.

CHAPTER-II

Review of Literature

Starch is the main component which constitutes 75% of the wheat kernel and has a direct affect on the nature and quality of flour and end products of wheat. Reduced starch content accounts for most of the reduction in grain dry matter at high temperature. Grain filling is mainly a process of starch biosynthesis and accumulation. Extensive studies have been done on the effects of heat stress on the activities of enzymes involved in sucrose to starch metabolism in cereals (Jenner, 1994; Cheih and Jones, 1995; Duke and Doehlert, 1996; Wilhelm *et al.*, 1999; Hurkman *et al.*, 2003 and Yang *et al.*, 2004). The attempts to increase starch content in cereals have a direct bearing on the food industry. Starch content can be manipulated by chemical means provided we have adequate information on the pathway of starch biosynthesis and the control mechanisms associated with it.

The relevant literature pertaining to "Starch metabolism and end use quality of durum wheat in response to high temperature stress"

has been reviewed in this chapter. The research work pertinent to the present study in India as well as abroad is reviewed under following sub-headings :

1. Whole plant response to high temperature.
2. Physiological parameters influenced by high temperature
3. Change in starch metabolism in response to high temperature
   a. Enzymes
   b. Metabolites
   c. Biochemical attributes
4. Grain quality characteristics in response to high temperature

1. Whole plant response to high temperature :

Both grain yield and quality are adversely affected by high temperature stress. High temperature during grain filling in wheat limits the grain yield (Zahedi *et al.*, 2003). Grain yield decreases by approximately 3-4% for each 1^0C rise in temperature above 15^0C during grain filling (Wardlaw and Wrigley, 1994; Bencze *et al.*, 2005) and this occurs primarily because starch accumulation is reduced by high temperature. As temperature increases above a mean of 15^0C during grain filling, the increase in the rate of starch deposition does not adequately compensate for the reduced duration of starch

accumulation (Sofield *et al.*, 1977b; Nicolas *et al.*, 1984) and consequently grain size and yield are reduced.

Zhong-Hu and Rajaram (1994) concluded that the grain filling rate was more temperature sensitive than days to anthesis and duration of grain filling. High temperature typically above 34^0C, affects final grain weight by reducing the duration of grain filling due to suppression of current photosynthesis (AI-Khatib and Paulson, 1984; Bencze *et al.*, 2004). Four-fold differences in wheat yield were found when data of favourable and high temperature field environments were compared (Midmore *et al.*, 1984; Shpiler and Blum, 1986; Zhong-Hu and Rajaram, 1994; Plaut *et al.*, 2004). These differences were much greater than yield reductions by high temperature under controlled conditions (Chowdhury and Wardlaw, 1978; Wardlaw *et al.*, 1989a). Temperature as high as $30/25^0$C decreased kernel number upto 22% and kernel weight by as much as 38% (Wardlaw *et al.*, 1989a). High temperature during kernel filling decreased wheat yield by reducing kernel weight (Warrington *et al.*, 1977; Tashiro and Wardlaw, 1990a; Stone and Nicolas, 1994). Elevated temperature during grain filling decreased starch deposition and therefore, adversely affected yield (Gibson and Paulsen, 1999).

Ferris *et al.* (1998) reported decline in grain yield by 315 g m^{-2} with increase of $10^{0}C$ in maximum temperature at anthesis (78 DAS) in wheat. The decline was mainly because of 40% reduction in number of grains per spike. The relative grain yield in high temperature treatment from booting, anthesis and grain filling was only 5.6, 16.6, 29.1% of control plants grown at $17/15^{0}C$ day/night in wheat (Kafi and Stewart, 1998). Grain weight seems to be less sensitive to heat stress than grain number (Abrol and Ingram, 1996).

Average wheat yield loss of 10-15% at moderately high temperature was mainly due to decreased kernel weight (Wardlaw and Wrigley, 1994). Under heat stress conditions, 1000-grain weight had the highest positive direct effect and significant positive correlation with grain yield while days to heading had high negative direct effect and significant negative correlation with grain yield in wheat (Munjal and Dhanda, 2004). Sarker *et al.* (2002) reported high positive correlation of harvest index, biomass and 1000-grain weight with grain yield in wheat.

2. Physiological parameters influenced by high temperature :

a) Chlorophyll and chlorophyll stability index :

High chlorophyll content is a desirable characteristics because it indicates a low degree of photoinhibition of the photosynthetic

apparatus, therefore reducing carbohydrate losses for grain growth. The genotypes WH 1022 had the highest chlorophyll content, it may account for a higher biomass accumulation (Dhanda *et al.*, 2007). Physiological evidence indicates that loss of chlorophyll during grain filling is associated with reduced yield in the field (Reynolds *et al.*, 1994). Studies in controlled environments have revealed genetic variability in photosynthetic rate among wheat cultivars when exposed to high temperatures (Wardlaw *et al.*, 1980; Blum, 1986). Such differences in photosynthesis under heat stress have been shown to be associated with a loss of chlorophyll and a change in the 'a:b' chlorophyll ratio due to premature leaf senescence (Harding *et al.*, 1990). Shukla *et al.* (1997) working on wheat genotypes under controlled environment reported increased grain chlorophyll under high temperature conditions. On the contrary, Ritcha *et al.* (1994) reported a decreased level of chlorophyll under high temperature. On an average chlorophyll 'a' decreased by 29%, chlorophyll 'b' 38% and carotenoids 12% due to high temperature in wheat.

b) Chlorophyll fluorescence :

Chlorophyll fluorescence analysis is a powerful technique to provide a sensitive indicator of stress conditions in plants (Maxwell and Johnson, 2000). It can also be used to estimate the activity of thermal energy dissipation in photosystem II, which protects photosynthesis

from the adverse effects of light and heat stress. For this reason, chlorophyll fluorescence has been proposed as a useful tool for screening durum and bread wheat for drought and high temperature stress (Moffat *et al.*, 1990). Fluoresence transient can be used to address the effects of heat on crop productivity. It has been shown that there is linear relationship between CO_2 assimilation efficiency and actual PS II efficiency (Genty *et al.*, 1989). High temperature decreased mean chlorophyll fluorescence by 42 and 11% in seedlings and maturing plants (AI-Khatib and Paulson, 1990). Effects of high light intensity (2000 μmole quanta $m^{-2}s^{-1}$) at low (4^0C), room (25^0C) and high (40^0C) temperature on chloroplast fluorescence kinetics of faba bean (*Vicia faba* L.) and spinach (*Spinacia oleracia* L.) leaves show that it decreased significantly with higher temperature. Quantum efficiency, relative performance index and drawing forces for energy fluxes for electron transfer decreased, but that for dissipation increased with higher temperature (Misra *et al.*, 2001). Changes in photochemical efficiency of PSII have been shown not only to affect plant growth, but also population biology via effects on seedling establishment and survival (Ball *et al.*, 1995).

Heat stress displayed multiple effects on PSII in wheat. The results revealed that effects of heat stress on PSII were characterized by

two declined domains of temperature, moderately elevated temperature (30-37.5^{0}C) and severely elevated temperature (higher than 37.5^{0}C). At moderately elevated temperature, no change in maximal efficiency of PSII photochemistry and photochemical quenching was observed. Numerous reports on changes in chlorophyll fluorescence parameters in heat stressed leaves conform PSII being the primary target of stress induced injury (Yordanov *et al.*, 1997b; Taub *et al.*, 2000; Shabala, 2003; Kouril *et al.*, 2004; Baker and Rosenquist, 2004). The decrease in dark-adapted Fv/Fm and increase in F_0 to indicate the occurrence of photoinhibitory damage in response to high temperature (Gamon and Pearcy, 1989). The chlorophyll fluorescence gives the potential to estimate photosynthetic performance, under conditions in which other methods would fail, in a manner that is almost instantaneous. PSII is also accepted to be the most vulnerable part of the photosynthetic apparatus to light-induced damage. Damage to PSII will often be the first manifestation of stress in a leaf.

c) Membrane thermostability :

High temperature stress leads to loss of membrane integrity followed by increase in cell permeability (Taiz and Zeiger, 2002). Membrane thermostability is an important index of screening cultivars for thermotolerance (Blum and Ebercon, 1981; Munjal *et al.,* 2004).

A cell membrane system that remains functional during heat stress especially during grain filling stage appears central to adaptation of plant to high temperature. Measurement of electrolyte leakage from leaf discs bathed in deionized water after exposure to heat shock treatment has been used as an indicator of membrane thermostability in response to heat stress. Large variations in relative injury were observed among genotypes tested at both seedling and anthesis stages (Saadalla *et al.*, 1990; Tahir and Singh, 1993; Reynolds *et al.*, 1994; Dhanda and Munjal, 2006)

This technique seems to be an efficient measure of heat tolerance and correlates well with tolerance of stress in other plant processes (Sullivan and Ross, 1979; Blum, 1988; Premchandra *et al.*, 1989 and Deshmukh *et al.*, 1991). The amount of electrolyte leakage is a result of membrane permeability, which is turn is a function of the degree of injury induced by the high temperature.

Levitt (1980) suggested that heat shock leads to an increased leakage due to transition in membrane lipid phase. A two minutes heat treatment at 47.5^{0}C in tobacco increased the leakiness of the membranes (Benzioni and Itai, 1972).

Kaur *et al.* (1988) compared ion leakage between WH 147 and its mutant WH 147 M at high temperature. They observed that the ion

leakage was relatively less in the mutant which also performed better under high temperature.

Saadalla *et al.* (1990) investigated the suitability of using the membrane thermostability (MT) test for ascertaining heat tolerance of winter wheat. They found that when winter wheat exposed to 48 h hardening and subjected to the MT test at 49^{0}C, the cultivars produced much greater differences in relative injury. Deshmukh *et al.* (1991) measured ion leakage in four drought susceptible and four tolerant wheat varieties and observed higher ion leakage in susceptible types as compared to tolerant ones.

Ibrahim and Quick (2001) determined the genetic control of heat tolerance through diallele analysis of selected wheat germplasm. Heat induced damage of plant membranes was assayed by the membrane thermal stability (MTS) assay, which measures electrolyte leakage from leaf tissue after exposure to high temperature. They suggested that leaf tolerance based on MTS can be improved using the existing genetic variability available within the germplasm.

d) Osmotic potential :

Osmotic adjustment may be defined as an increase in the number of osmotically active solute particles in cells as a response to environmental stresses.The major advantage of osmotic adjustment is

the maintenance of positive turgor as water deficit develops (Hsiao *et al.*, 1976; Turner and Jones, 1980). Wheat is one of the best documented species with an ability for osmotic adjustment (Simmelsgaard, 1976; Blum *et al.*, 1983; Morgan, 1980, 1988; Johnson *et al.*, 1984). Increased transpiration due to dehydration induced osmotic adjustment in detached flag leaves of durum wheat while prolonged saturation without dehydration had no significant effect on osmotic potentials (Kikuta and Richter, 1988). Stress-induced decrease of osmotic potential in *Triticum aestivum* has been reported to be mainly due to active accumulation of sugars (Munns and Weir, 1981; Johnson *et al.*, 1984) and amino acids (Munns *et al.*, 1979; Drossopoulos *et al.*, 1985). In general, increase in all amino acids was observed in *T. durum* under water and salt stress, only the proline content was high enough to be considered the principal solute in osmoprotection. The accumulation of proline during water deficit may have other functions, such as enzyme protection and stabilization of biological membranes; the degradation of proline may improve the energy status of cells recovering from water deficit (Saradhi and Saradhi, 1991). The estimates of flag-leaf osmoregulation were derived from relationships between osmotic potentials and relative water contents. The measure of osmoregulation being the predicted value of the relative water content corresponding to an osmotic potential of -2.5 MPa. In low

osmoregulating genotypes, osmotic potential declined from -0.6±0.1 MPa at full hydration to -1.2±0.2 MPa at zero turgor pressure. However, in high osmoregulating genotypes, the fall in osmotic potential matched the fall in water potential from a value of -0.5±0.1 MPa at full hydration to a water potential of -1.3 MPa, after which turgor pressure declined to zero at a water potential of -2.0±0.1 MPa (Morgan, 1988). The high temperature stress and drought reduced osmotic potential in wheat and sorghum (Machado and Paulsen, 2001). The critical value of osmoregulation for the durum wheat lines was lower at 65-68%. The average maximum yield grain with high osmoregulation of 11-17% in bread wheat and 7% in durum wheat (Morgan *et al.*, 1986). Merah (2001) reported the positive correlations between relative water content (RWC), osmotic potential and both grain yield (GY) and harvest index (HI).

e) Dry matter accumulation in grain :

Temperature is a major factor affecting the growth and development of wheat during early phase of floret formation, grain set and their development. Temperature also brings about changes in sink size and influences the total dry matter production and harvest index (Shukla *et al.*, 1988). Undoubtedly, the grain sink potential is genetically determined, the capacity of grain to develop is a matter of competition for space or assimilate supply which is directly linked to

the thermal environment during grain growth and development (Gupta *et al.*, 2002). Ritcha *et al.* (1994) reported an increased activity of ADP-glucose linked starch synthetase under high temperature during the active dry accumulation period and has linked it with temperature tolerance in developing grains of wheat cv. Kalyansona.

3. Change in starch metabolism in response to high temperature :

Starch and sucrose are the major end-products of photosynthesis. Carbon fixed during photosynthesis is either retained in the chloroplast and converted to the storage starch or transferred to the cytosol in the form of triosephosphates and converted to sucrose which is finally converted to starch in the amyloplasts.

The major substrates for sucrose synthesis in photosynthetic tissues are three carbon sugar phosphates. They are exported from the chloroplast in the form of triose phosphates. The pool of triose phosphate, dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP) is maintained at equilibrium within the cytoplasm by triosephosphate isomerase. A subsequent reaction involves an aldol condensation of DHAP and GAP, catalysed by the enzyme aldolase, to form fructose-1, 6-bisphosphate (F-1, 6-BP). A phosphate group is then cleaved from the C1 carbon by a specific phosphatase, fructose-1, 6-bisphosphatase (FBPase) to form

fructose-6-phosphate (F-6-P). Glucose-6-phosphate (G-6-P) and glucose-1-phosphate (G-1-P) are maintained in equilibrium with F-6-P pool by the action of phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM), respectively. Uridine triphosphate (UTP) and G-1-P catalyzed by the enzyme UDPG-pyrophosphorylase (UGPase). UDPG is then combined with F-6-P to form sucrose-6-phosphate (S-6-P), catalysed by sucrose phosphate synthase (SPS). The next reaction in the pathway is catalyzed by sucrose-6-phosphate phosphatase (SPPase), which catalyzes the hydrolysis of S-6-P to sucrose. By the action of sucrose synthase, sucrose is converted to fructose and UDP-glucose (UDPG). Fructose forms F-6-P, catalysed by fructokinase, which ultimately gives rise to G-1-P. UDPG also gives G-1-P by the action of UDP glucose pyrophosphorylase (UGPase). G-1-P moves into the amyloplast where by the action of AGPase forms ADP-glucose which ultimately is channelised into starch, by starch synthase. Pathway of starch biosynthesis is shown in Fig. 1.

a) Enzymes :

High temperature during grain filling period in wheat limits grain yield (McDonald *et al.*, 1983; Wrigley *et al.*, 1994). Reduction in grain weight at high temperature is mainly due to its effects on starch deposition (Bhullar and Jenner, 1985), because the supply of

assimilates to the grains at elevated temperature does not seem to be the major limitation for starch production (Chowdhury and Wardlaw, 1978; Wardlaw *et al.*, 1980; Nicolas *et al.*, 1984). The conversion of sucrose to starch is impaired at high temperature and limits starch synthesis (Bhullar and Jenner, 1985). At temperatures above 30^{0}C (in the high temperature range (HTR) between 30 and 40^{0}C), even for short periods, the rate of starch deposition is slower than that to lower temperatures. This response is attributable to a reduction in the activity, possibly due to thermal denaturation, of a enzymes involved in starch synthesis. The major enzymes involved in starch synthesis is shown in Fig. 2.

Among the enzymes involved in the pathway of sucrose to starch in wheat endosperms, soluble starch synthase (SSS) is the most sensitive to high temperature (Hawker and Jenner, 1993; Jenner *et al.*, 1993; Keeling *et al.*, 1993), and it has an unusually low optimum temperature for maximum activity (Denyer *et al.*, 1994). Below 30^{0}C, however, the loss of the activity of soluble starch synthase alone is not large enough to account in the responses of starch deposition to rising temperatures (Rijven, 1986). Several forms of soluble starch synthase are found in cereal endosperm, and some forms may be more tolerant to high temperature than others (Jenner, 1994). Other kinetic

properties of SSS may account for the temperature response of starch synthesis in the range 20-30^{0}C (Jenner *et al.*, 1995; Jenner and Sharma, 1997).

The first unique steps in starch biosynthetic pathway is catalyzed by AGPase, which forms ADP-glucose and inorganic pyrophosphate (PPi) from G-1-P and ATP. AGPase is present in both source (leaves) and sink tissues (grains). It was reported to be restricted into plastids in photosynthetic tissues (Preiss, 1991). High temperature stress affects starch biosynthesis by decreasing AGPase activity. AGPase was irreversibly inactivated when exposed to temperatures above 40^{0}C in wheat (Kanchan, 2004). Heat stress affects AGPase more than any other enzymes, which may contribute to reduction in the efficiency of starch synthesis (Singletary *et al.*, 1994). The response of AGPase is, in someways, opposite of soluble starch synthase in response to high temperature. Work by Duke and Doehlert (1996) involving heat stressed kernels of maize also demonstrated that AGPase activity is constricted more than several other enzymes of sugar metabolism. The reductions in starch accumulation generally do not occur without a decrease in AGPase activity of 40% or more (Singletary *et al.*, 1997). Cyanobacterial AGPase is irreversibley inactivated when exposed to a temperature above 40^{0}C (Gomez *et al.*, 2000). There was 28%-50%

decrease in AGPase and 40-50% decrease in sucrose synthase activity measured under optimal conditions in extracts from potato tubers exposed to high temperature (Lafta and Lorenzen, 1995). In potato tubers, an 80-95% reduction of AGPase expression led to decreased rates of starch accumulation (Muller-Rober *et al.*, 1992) whereas recent studies in potato tubers indicate that reducing soluble starch synthase to 20% of the wild-type level has no effect on starch accumulation (Marshall *et al.*, 1996). This indicates the importance of AGPase rather than soluble starch synthase in controlling the rate of starch synthesis under normal grown conditions in potato tubers.

In tubers incubated at temperature above 30^{0}C, the overall activities of sucrose synthase and AGPase declined slightly, whereas soluble starch synthase remains unchanged. (Kraus and Marschner, 1984). Elevated temperature led to an activation of sucrose phosphate synthase involving a change in its kinetic properties. There was a strong correlation between sucrose phosphate synthase activation and the *in vivo* level of G-6-P.

The sucrose-phosphate synthase (SPS) is the enzyme responsible for sucrose synthesis in the non-green tissues. The conversion of sucrose to starch is impaired at high temperature and limits starch synthesis (Bhullar and Jenner, 1985). High temperature had no effect on the amounts of sucrose in the endosperm of Kavko (tolerant) and

Lyallpur (sensitive) wheat cultivars. ADP-glucose is produced from sucrose in the endosperm and is the immediate substrate for starch synthesis. But Zahedi *et al.* (2003) observed no correlation between the changes in sucrose and ADP-glucose concentrations at high temperature.

Decreases in the activities of AGPase has been observed both in maize kernels and wheat endosperms under heat stresses, thus resulting in a reduction of starch synthesis (Keeling *et al.*, 1993; Wilhelm *et al.*, 1999).

The activity of ADP-glucose pyrophosphorylase was reduced by upto 35% in the endosperm of grains heated at 35^{0}C (Hawker and Jenner, 1993) and while the activity of sucrose synthase was scarcely affected, the mass-action ratio of the sucrose synthase reaction was perturbed at high temperature (Jenner, 1991). The activities of other enzymes in the pathway, including the branching enzyme, were not significantly affected by exposure to a temperature of 35^{0}C (Hawker and Jenner, 1993).

A disaccharide sucrose and its cleavage products glucose and fructose are central molecules for carbohydrate metabolism. Invertases mediate the hydrolytic cleavage of sucrose into the hexose monomers i.e. glucose and fructose. Plants possess 3 type of invertases, which are

located in apoplast, cytoplasm and the vacuole. Invertase involved in carbohydrate partitioning, development processes and in biotic, abiotic interactions with other plant hormones (Roitsch and Gonzalez, 2004). Sidhu and Singh (2002) reported that activity of soluble acid invertase in wheat was higher in photosynthetic structures above flag leaf node such as peduncle, rachis and bracts, during early stages of grain growth, but reverse was true for soluble neutral invertase activity. Neutral and alkaline invertases are confined to mature tissues and are involved in intracellular glucose and fructose levels. In young Vicia embryos, invertases in maternal tissue control both the concentration and composition of sugars (Borisjuk *et al.*, 2004). Of all the invertases, cell wall invertase activity was higher 44-110% than soluble invertase in young leaves, indicating apoplastic unloading of imported sucrose as major route. Minhas *et al.* (2003) observed that as leaves develope to become net exporters of carbohydrate, the activity of sucrose degrading enzymes declined by 90%.

b) Metabolites :

Much of the work concerned with the biosynthesis of starch in developing cereal grains is done with determination of *in vivo* concentration of both substrates and products simultaneously with measurement of relevant enzyme activities (Kumar and Singh, 1984; Truesdale *et al.*, 1999; Zahedi *et al.*, 2003). They reported that such

studies might help in understanding the control mechanisms associated with the pathway of starch biosynthesis and thus provide chemical means to manipulate starch content vis-a-vis grain yield.

High temperature stress alters the level of metabolites and corresponding enzymes. Decrease in the level of 3-PGA due to inhibition in activities of AGPase under elevated temperature is one such example (Geigenberger *et al.*, 1998).

Zahedi *et al.* (2003) reported that the concentration of ADP-glucose was lower in the grains of Kavko (high temperature tolerant) than in those of Lyallpur (high temperature sensitive) and the amount of ADP-glucose in both wheat cultivars was lower at 30/25^{0}C than at 20/15^{0}C. ADP-glucose is produced from sucrose in the endosperm and is the immediate substrate for starch synthesis. There was no correlation between the changes in sucrose and ADP-glucose concentrations at high temperature.

The amount of ADP-glucose decreased in potato tubers when exposed to elevated temperature. There was a strong correlation between *in vivo* levels of 3-PGA and ADP-glucose in tubers incubated at different temperature and the decrease in ADP-glucose correlated with the decrease in the starch content.

The level of ADP-glucose increased slightly when the temperature was increased to 25^0C. ADP-glucose decreased by 23% and 45% in tubers incubated at 30^0C and 37^0C, respectively which corresponds to the inhibition of starch synthesis at these temperature. As the temperature was increased from 19^0C to 37^0C, G-6-P level increased progressively from 140-290 n mol g^{-1} fresh weight. No consistent changes were observed in the levels of ATP, ADP, UDPG or PPi. Increasing the temperature from 16^0C/21^0C to 25^0C/35^0C (night/day) in wheat resulted in a decrease in the level of metabolites (hexose phosphates, UDP-glucose, ADP-glucose) (Jenner, 1991). UDP-glucose, ADP-glucose decreased more gradually during the entire time interval, being significantly reduced by 28% and 13%, respectively compared with low temperature control. The overall decrease in metabolite levels in wheat during long-term exposure to heat indicates that there may be additional factors besides an inhibition of starch synthase that contribute to the small increase in starch deposition with increasing temperature (Geigenberger *et al.*, 1998).

Keeling *et al.* (1993) concluded that metabolism of sucrose through pentose phosphate pathway and glycolysis and the conversion of sucrose into starch are temperature dependent processes.

The amount of G-6-P was over 25 times higher than that of G-1-P and about 5 times higher than that of F-6-P throughout the period of grain development. The level of metabolites increased during grain development, reaching maximum at 21 DAA and declining thereafter. Both the nucleotide sugars were present in minute quantities at this stage. Levels of PPi were also monitored by coupling with UDPG pyrophosphorylase, but it could not be detected at any of the stages. The ratios of the level of G-6-P : F-6-P : G-1-P and UDPG : ADPG dose to 25 : 5 : 1 and 3 : 1, respectively (Kumar and Singh, 1984).

The conversion of fructose, released from sucrose synthetase reaction, to G-1-P has been reported to be present in developing grains of barley (Baxter and Duffus, 1973a; Batra and Mehta, 1981b), maize (Tsai *et al.*, 1970) and rice (Perez *et al.*, 1975) soon after anthesis. In developing cereal grains, activity in general, increases from very low values just after anthesis to a peak and then declines (Perez *et al.*, 1975; Batra and Mehta, 1981b) towards maturity. G-1-P then gets converted to nucleotide sugars, ADPG (UDPG) by AGPase.

F-2,6-P_2 plays a major role in regulating partitioning between sucrose and starch synthesis during photosynthesis. In the leaves of C_3 plants, F-2,6-P_2 regulates the partitioning of CO_2 fixed during photosynthesis between starch and sucrose via inhibition of the

cytosolic form of F-1, 6-bisphosphatase and inhibit sucrose synthesis and stimulate the synthesis of starch (Truesdale, 1999).

c) Biochemical attributes :

Starch and protein are the major constituents of mature wheat grain. In *Triticum durum*, the starch content ranged from 58 to 72%, the total sugar ranged from 0.66 to 1.60%.

Bhullar and Jenner (1985) demonstrated the reduction in amounts of both starch and protein with increase in temperature to 30^{0}C but the amount of starch seems to be reduced relatively more than that of protein.

Starch in the wheat grain is deposited in two distinct types of granules : large A-type granules which are initiated early during the development of the endosperm and smaller B-type granules which are initiated later than the A-type. Under conditions of heat stress (Hoshikawa, 1962; Moss, 1963) fewer B-type granules are initiated and the size of the A-type granules are reduced, but number of A-type granule is not reduced. So, the reductions in the weight of starch as a result of high temperature attributable to the reductions in numbers of B-type granules. Numerous studies involving maize (Jones *et al.*, 1984; Keeling *et al.*, 1994; Singletary *et al.*, 1994), wheat (Bhullar and Jenner,

1985) and barley (Macleod and Duffus, 1988) have shown a negative effect of heat stress on starch deposition in the kernel.

As temperature increase above 20^{0}C during grain filling, rate of starch deposition does not adequately compensate for reduced duration of starch accumulation and consequently reduced grain size and yield in wheat (Prakash *et al.*, 2003).

High temperature exerts its effects by disturbing sucrose : starch balance in wheat (Bhullar and Jenner, 1985). High temperature declined photosynthesis rate, soluble sugars content and water use efficiency in wheat (Shah and Paulson, 2005). Ultrastructural changes in maize endosperm that result from exposure to high temperature during cell division include presence of an irregular shaped nucleus, altered size of the nucleolus, highly dense nucleoplasm and a decrease in the number of proplastids and amyloplasts. Thus, the endosperm cavity was not filled, the total number of starch granules decreased by 45 and 80% after exposure to 4 and 6 days of high temperature treatment, respectively. This also resulted in a 35-70% reduction in total starch accumulation (Commuri and Jones, 1999) by relative stability of granule bound starch synthase (GBSS) at moderately high temperatures may explained the increase in the percentage of amylose in the grains (Shi *et al.*, 1994; Tester *et al.*, 1995).

All or most GBSS activity survived at the high temperature treatment, high temperature nevertheless reduced amylose synthesis almost as much as amylopectin synthesis (Zahedi, 2001) indicating that the synthesis of these two components is interdependent. So, there is progressive increase in the grain amylose percentage over the grain-filling period, especially at high temperature (Zahedi *et al.*, 2004). The components of starch were reduced at high temperature, amylose by 38% and amylopectin by 40% (Zahedi, 2001).

The branch chain pattern of amylopectin is changed by temperatures and it is due to reduced activity of branching enzyme at high temperature (Jiang *et al.*, 2003). Increase in the granule bound starch synthase I (GBSSI) activity at low-temperature presumably would cause the accumulation of more amylose during grain development, thus reducing grain quality of rice (Suzuki *et al.*, 2002).

4. Grain quality characteristics in response to high temperature :

Quality of food grain is a complex phenomenon and may be influenced by several factors which may be genetic and/or environmental. Cultural practices also considerably influence the grain quality.

Singh and Paliwal (1986) studied 13 wheat varieties for physical characteristics and observed that 1000-kernel weight ranged from 40.3

to 53.2 g, grain hardness from 9.36 to 13.25 kg/grain. Hooda (2002) studied some physical parameters of wheat variety WH 423 and observed 41.50 g 1000-grain weight, 8.45 kg/grain hardness. Hadded *et al.* (1997) studied the physical parameters of the grains, nutritional composition and quality of wheat flour of *T. aestivum* and *T. durum* cultivars. The cultivars AKW 381 and HD 2380 recorded highest 1000-grain weight, whereas highest gluten and protein content was observed in durum cultivar MAC 59. The protein content in grains ranged from 11.99 to 13.80%, dry and wet gluten content ranged from 8.88 to 10.91% and 27.60 to 35.18%, respectively. Sharma (2000) evaluated four wheat varieties namely WH 542, Sonak, WH 553 and UP 2338 and reported that 1000-grain weight and grain hardness of wheat ranged from 33.0 to 41.8 g and 8.5 to 10.7 kg/grain, respectively. Singh (2004) reported that 1000-grain weight of wheat ranges from 31.90 to 53.03 g and grain hardness from 9.36 to 13.9 kg/grain.

Madan *et al.* (2006) investigated that soluble protein contributed upto 30% of total crude protein in mature grains. However, at early stages of development proportions of soluble proteins ranged from 16 to 25% of total proteins. This is due to the fact that initially grain protein almost entirely composed of insoluble polymers (glutenins) as shown by Carceller and Aussenoc (1999). Grain protein accumulation declines as the temperature increases, although it is usually less sensitive than

starch. As a result, increased temperature during grain filling tends to increase grain protein percentage (Randall and Moss, 1990; Rao *et al.*, 1993). In many varieties of wheat, the ratio of glutenin and gliadin decreases with temperature above 30^0C (Stone and Nicolas, 1996; Stone *et al.*, 1996). It appears that glutenin/gliadin ratio decreases because the accumulation of gliadin is reduced less by elevated temperature than that of glutenin (Stone and Savin, 2000). High temperature stress (>35^0C) during the grain filling period has the potential to modify grain quality. Total soluble protein is also reduced by high temperature. This might be due to protein denaturation and inhibition of protein synthesis at higher temperature, since the injury from high temperature has often been attributed to the denaturation of proteins (Blumenthal *et al.*, 1995a). Numerous studies involving maize (Jones *et al.*, 1984; Keeling *et al.*, 1994; Singletary *et al.*, 1994), wheat (Bhullar and Jenner, 1985) and barley (MacLeod and Duffus, 1988) have shown a negative effect of heat stress on starch deposition in the kernel. The protein content and the grain hardness feature judge the type of end produce that can be made out of the grain.

In addition to the reduction of kernel weight and size, physiological stress at elevated temperature was also evident in the appearance of kernel's surface. Generally, wheat kernels filled at 40^0C

showed a wrinkled surface because of shriveling. It is known that cell walls are formed in the endosperm four days after anthesis and that a period of rapid cell division, with cells filling the embryo sac, occurs six days after anthesis. High temperature soon after fertilization may result in abnormal cell division, which may explain the production of shrunken kernels (Tashiro and Wardlaw, 1990a).

In normal wheat grains, the sedimentation value ranged from 21 to 47 ml with a mean value of 34 ml (Misra and Gupta, 1995). During heat stress, the SDS sedimentation volumes either became higher or remained at similar levels (Bencze *et al.*, 2004). Comparing the nutritional quality of bread and durum wheat samples, Madan *et al.* (2006) reported that the β-carotene contents were distinctly higher in *T. durum* (5.02 ppm) than those in *T. aestivum* (3.22 ppm) ranging 4.05 to 7.42 ppm and 1.87 to 4.28 ppm, respectively.

Shi *et al.* (1994) demonstrated that hardness varied inversely with increasing growing temperature and further reported that wheat hardness depends upon the starch-protein interaction that weaken at elevated temperature during grain-filling period and thereby decrease kernel hardness.

CHAPTER-III

Materials and Methods

EXPERIMENTAL MATERIALS

Investigations were carried on durum wheat cvs. WH-896 and WH-912. Seeds of durum wheat used in the experiment were obtained from wheat section, Department of Plant Breeding, College of Agriculture, CCS Haryana Agricultural University, Hisar.

EXPERIMENTAL DETAILS

Plants of both the varieties of durum wheat were raised in polythene bags containing 5 kg of dune sand in screen house.

Date of sowing was 15th November, 2005. Thinning was done after one week and only three plants of comparable growth per pot were maintained. Irrigations were given at 50 per cent depletion of the available soil moisture calculated on the basis of water lost. Complete Hoagland nutrient solution (150 ml) was supplied to each bags five times before heading.

Starch metabolism and end use quality of durum wheat in response to high temperature stress

Table 1 : Brief description of genotypes used in study :

	WH 896	**WH 912**
Days to heading	104 (Late)	109 (Late)
Days to maturity	142 (Late)	137 (Medium)
Plant height (cm)	87 (Medium)	83 (Medium)
Foliage colour	Dark Green	Green
Spike length (cm)	8.49 (Medium)	7.62 (short)
Average yield (t/ha)	4.2	4.7

Chemical composition of the Hoagland nutrient solution (Arnon and Hoagland, 1940) :

Major salt		
Stock solution 1	Ca $(NO_3)_2$	364.0 gL^{-1}
Stock solution 2	KNO_3	221.3 gL^{-1}
Stock solution 3	$MgSO_4$	217.6 gL^{-1}
Stock solution 4	KH_2PO_4	62.1 gL^{-1}
Micronutrients		
Stock solution 5	$ZnSO_4$	0.097 g
	H_3BO_3	1.269 g
	Na_2HPO_4	0.400 g
	$CuSO_4.5H_2O$	0.035 g
	$MnSO_4$	0.609 g
	Volume with water	1 L
Iron Source		
Stock solution 6	Tartaric acid	4 gL^{-1}
	$FeSO_4.7H_2O$	5 gL^{-1}

Nutrient solution 25 L was prepared by mixing 62.5 ml each of stock solutions 1 to 5 and 15 ml of stock solution 6.

TREATMENTS

a) Control (ambient temperature)

b) High temperature stress (by shifting the bags to polyhouse with maximum temperature 5-9^{0}C> than ambient). The plants were kept there upto grain maturity.

Minimum and maximum temperature were recorded during treatment period both under screen house and polyhouse conditions. High temperature stress quantification was done by calculating heat degree days (HDD) as reported by Rane and Nagarajan (2004). HDD is expressed as sum of daily mean temperature during crop growth period (1st day after sowing to physiological maturity).

Heat degree days	Control	High temperature	Difference
	852.5	1050.5	198.0

SAMPLING

The plants were sampled weekly from two weeks after anthesis to physiological maturity stage. Grains from middle portion of ear were used for analysis.

A) Physiological parameters :

Chlorophyll :

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were estimated by Dimethyl Sulphoxide (DMSO) method of Hiscox and Israelstam, 1979.

One hundred mg of flag leaf tissue was taken in 15.0 ml of DMSO in a test tube and kept for 3 hours at $60\pm1^{0}C$ in an oven. After cooling

absorbance was measured at 454, 645, 665 nm against DMSO as a blank.

Chlorophyll were estimated using following equation :

Chlorophyll a μg ml^{-1} = $(12.19A_{665} - 3.45\ A_{645})$

Chlorophyll b μg ml^{-1} = $(21.99\ A_{645} - 5.32\ A_{665})$

Carotenoids μg ml^{-1} = $[1000\ A_{454}$ – 2.86 x Chl. (a) – 129.9 x Chl. (b)]/221

$$\text{Chlorophyll 'a' content } (\mu \text{ mol g}^{-1}) = \frac{\text{Chl. (a) x Volume of DMSO (15ml) x 1.119}}{\text{Weight of leaf (100 mg)}}$$

$$\text{Chlorophyll 'b' content } (\mu \text{ mol g}^{-1}) = \frac{\text{Chl. (b) x Volume of DMSO (15ml) x 1.102}}{\text{Weight of leaf (100 mg)}}$$

$$\text{Carotenoid content } (\mu \text{ mol g}^{-1}) = \frac{\text{Carotenoid x Volume of DMSO (15ml) x 1.809}}{\text{Weight of leaf (100 mg)}}$$

Chlorophyll fluorescence :

This was recorded using CID chlorophyll Fluorometer OS-30p (USA Made).

Chlorophyll stability index (CSI) :

For estimation of chlorophyll stability index (CSI), another 100 mg leaf tissue from middle portion of flag leaf was taken in a test tube. These were heated at 49^0C for 30 min in a water bath. After cooling, 15.0 ml of DMSO was added and processed for extraction of pigments as explained above. The CSI was calculated as follows :

$$\text{CSI (\%)} = 1 - \frac{\text{Total chlorophyll of heated samples}}{\text{Total chlorophyll of non-heated sample}} \times 100$$

Membrane thermostability :

This was measured according to method of Ibrahim and Quick (2001). One hundred mg of fresh leaf tissue was taken from the middle

portion of flag leaf and washed twice with deionised water and then incubated in controlled temperature water bath at 49^0C for 30 min.

At each sampling, 10 ml of deionized water was added to treated vials. Vials were held at 10^0C for 18 to 24 h to allow diffusion of electrolytes from leaf. Then vials were brought to 25^0C and shaken, initial conductance of vial contents was determined with an electrical conductivity meter. Vials were then placed in an autoclave at 0.10 MPa pressure for 10 min to completely kill the leaf tissue and release all the electrolytes. The level of MTS was determined from the following calculation :

$$MTS\ (\%) = (1 - T_1/T_2) \times 100$$

Where, T_1 and T_2 refer to conductance value before and after autoclaving, respectively.

Osmotic potential :

Osmotic potential of flag leaf was determined by Psychrometric technique using a model 5100-B vapour pressure osmometer (Wescor Inc., Lorganan, Utah, USA). The leaves were excised from the plant between 10:00 AM and 12:00 noon and were sealed in an Ependoff tubes individually and quickly frozen at -10^0C. Before measuring the osmotic potential the samples were thawed for 60 min at 25^0C. The

tissue was then pressed with a flat glass – rod to take out the sap. Then a filter paper disc was dipped in the sap and immediately placed in the chamber of vapour pressure osmometer and the chamber was sealed. After about 2 min the osmotic potential readings were displayed on the digital meter automatically, which were recorded. The osmometer was calibrated by using standard solution of NaCl and the calculations were made as following :

40 m osmo = -1 bar

-10 bar = 1 MPa

Dry matter accumulation in grain :

The separated 500 mg of grains from middle portion of the spike were wrapped in paper-sheets and allowed to dry at 70^0C for 2 days and finally at 90^0C for 4 hours. The dry weight was measured by weighing the dried grain.

B) Quality characteristics :

Grain shriveling score (%) : The grain shriveling score was observed visually and it was recorded from 100 grains for each variety.

Grain hardness (kg/seed) : Grain hardness was measured by pressing ten average sized well fitted grains in the grain hardness tester (Manufactured by Kiya Seisakusho Ltd., Japan). The force was applied

to crush the grains by turning the knob. The force (kg) displayed on dial at the time of crushing the grain was recorded.

Grain weight/spike : The grains were weighed to obtain grain weight/spike for each of the three replicates. Final reading represented as mean of three replicates.

Grain number : Grains were counted for each of the three replicates in both varieties.

Test weight : Test weight was recorded by weighing 1000-grains for each variety.

C) BIOCHEMICAL ATTRIBUTES

Total soluble carbohydrate (TSC) :

Extraction :

One hundred mg of dry grain (flour) were grinded and refluxed in 10 ml distilled water for 2-3 h. It was cooled and centrifuged at 10,000 x g for 30 min. The supernatant was kept aside and pellet was refluxed in 10 ml distilled water again for 1 h and then centrifuged. The supernatants were pooled and pellet was refluxed again in 5 ml distilled water for 30 min and centrifuged. The total volume was made to 25 ml with distilled water. This constitutes the soluble carbohydrate fraction. The pellet was used for starch extraction.

Estimation of total soluble sugars :

Total soluble sugars were estimated by the method of Yemm and Willis (1954).

Procedure :

Five ml of anthrone reagent (0.2% H_2SO_4 v/v) was pipetted in test tubes (150 x 25 mm) and chilled in ice cold water. One ml of the sugar solution was layered on it and cooled for further 3-5 min. The content of the tubes was then thoroughly mixed while still immersed in ice cold water. The tubes, were closed with rubber stoppers bearing small capillary tubes and heated in a vigorously boiling water bath for 10 min and then cooled immediately in ice cold water. The absorbance of colored solution was obtained on a Spectronic-20 at 625 nm using reagent blank.

The amount of sugar present was then determined by referring to a standard curve prepared using glucose solution (20-100 μg ml^{-1}). In order to minimize the error, if any, arising from variations in heating time and the anthrone reagent used, standard sugar solution (50 μg ml^{-1}) was taken as standard with every set of estimation.

Starch extraction :

The pellet left after total soluble carbohydrate extraction was treated with 3 ml mixture of 6.5 ml of 52% perchloric acid + 5 ml of distilled water and centrifuged at 10,000 xg for 30 min. The

supernatant was kept aside and pellet again treated two times with the same reagent. The supernatants were pooled and total volume was made to 12.0 ml with distilled water and this constitutes starch fraction.

Estimation of starch :

Starch was estimated using Yemm and Willis (1954). To 50 μl aliquot of perchloric acid extract, various reagents and steps used for estimation of total soluble carbohydrate (TSC) were followed. Concentration of starch was calculated by multiplying the glucose standard values by 0.9 (0.9 g of starch on hydrolysis with perchloric acid yields one g of glucose).

Reducing sugars :

Estimated by method of Nelson (1944) as modified by Somogyi (1945).

a) Copper reagent A :

Twenty five g of anhydrous sodium carbonate, 25 g of potassium sodium tartrate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate in 800 ml of water, diluted to 1 l.

b) Copper reagent B :

Fifteen g of $CuSO_4.5H_2O$ was dissolved in 100 ml of distilled water containing 1-2 drops of conc. H_2SO_4.

c) Alkaline copper reagent :

It was prepared freshly by mixing reagents A and B in a ratio of 25:1 immediately before the use.

d) Arsenomolybdate reagent :

Twenty five g of ammonium molybdate was dissolved in 450 ml distilled water and 21 ml conc. H_2SO_4 was added with stirring. Three g of $NaHASO_4.7H_2O$ dissolved in 25 ml of water, was added with continous mixing and the solution was kept in incubator at 37^0C for 24 hours. Before use stored in Brown bottle.

Procedure :

One ml of test extract was taken in a test tube graduated upto 25 ml and 1 ml of copper reagent was added. The solution was then mixed, covered with aluminium foil and heated in a boiling water bath. After 20 min, the test tubes were cooled to room temperature and 1 ml of arsenomolybdate reagent was added. The contents were mixed thoroughly and diluted to 25 ml. In blank, 1 ml water was taken in place of sugar solution. Absorbance of the stable blue colour was read at 520 nm on a Spectronic-20. The amount of reducing sugars are then

determined by referring to a standard curve of glucose as the standard (20-100 μg ml^{-1}).

Non-reducing sugars :

The content of non-reducing sugars was calculated from the difference between the concentration of total sugars and that of reducing sugars.

Sucrose :

Sucrose was estimated by method of Johnson *et al.* (1964) with slight modifications.

Five ml of total soluble sugars extract was incubated with 1 ml of 0.1% invertase in presence of acetate buffer (pH 4.5) at 35^{0}C for 8 h. Reducing sugars in the hydrolysed extract were then determined. The reducing sugars due to sucrose were calculated and sucrose determined by the formula :

Sucrose = Increase in reducing sugars x 0.95

Amylose :

Amylose was estimated by the method of Williams *et al.* (1970).

Reagent :

a) Two g potassium iodide and 200 mg resublimed iodine were dissolved in a 50 ml of water and then volume was made to 100 ml.

b) Iodine reagent : Ten ml solution (a) was further diluted to 100 ml with distilled water.

Procedure : Twenty mg of grains (first dry the grains at room temperature and then in oven) samples grinded in pestle – mortar and put into 100 ml conical flask. Add 10 ml of 0.5 N KOH (28.05 g l^{-1}) in it and homogenize the mixture for 3-5 min and then kept aside for 1 h without disturbing it. Volume was then made to 100 ml with distilled water.

Estimation : To 10 ml aliquot, 5 ml of 0.1 N HCl (8.72 ml/l) and 0.5 ml of Iodine reagent was added and volume was made to 50 ml. The blue colour was developed. After 30 min, the intensity of blue colour developed was measured at 590 nm. The amount of amylose are then determined by referring to a standard curve (20-150 μg ml^{-1}).

Amylopectin :

The content of amylopectin was calculated from the difference between total starch and amylose content.

β-carotene :

β-carotene was estimated by the method of AOAC (1990).

Reagents :

Water saturated n-butanol – A solution of n-butanol and water in a ratio of 6:2 (v/v) was mixed and shaken vigorously. It was then allowed to stand till it separated into two phases, the upper clear layer was saturated n-butanol.

Extraction :

Ten g of powdered grains was dispersed in 50 ml water – saturated n-butanol to make a homogenous suspension. It was shaken gently and allowed to stand overnight (16 h) at room temperature in dark. The suspension was again shaken and filtered through Whatman Filter Paper No. 1 and the volume of filtrate was made 100 ml.

Procedure :

The absorbance (A) of the clear filtrate was measured at 440 nm in Spectronic-20 using saturated n-butanol as a blank. The amount of -β-carotene was calculated from the following equation :

$$\beta\text{-carotene content } (\mu g\ g^{-1}) = 0.0105+23.5366 \times A_{440}$$

Sedimentation value : Determined by the method of Axford *et al.* (1979).

Reagent :

SDS/Lactic acid reagent – This reagent was prepared by mixing 20% SDS (w/v), 80% Lactic acid (v/v) and distilled water in ratio of 20:1:8.

Procedure :

Whole meal (6 g) was added to 50 ml water in a 100 ml cylinder, a stopcock set going and the material dispersed by rapid shaking horizontally for 15 sec. The contents of the material were again shaken for 15 sec at 2 min and 4 min. Immediately after the last shake, 50 ml of SDS lactic acid reagent was added and mixed in by inverting the cylinder 4 times. Inversion (four times) was repeated at 6, 8 and 10 min. The contents of the cylinder were allowed to settle for 20 min (whole meal) before the sedimentation volumes were read.

Total nitrogen (crude protein) :

Total nitrogen was estimated by micro-Kjeldahl method (AOAC, 1990).

Reagents :

a) Digestion mixture (K_2SO_4 and $CuSO_4$ in the ratio of 9:1).

b) Nitrogen-free conc. H_2SO_4.

c) 40% NaOH solution

d) N/100 NaOH solution

e) N/100 H_2SO_4 solution

f) Methyl red indicator (0.1 g of the indicator dissolved in 60 ml of alcohol and water added to make the volume 100 ml)

Procedure :

a) Digestion : Sample (0.2 g) was taken in Kjeldahl flask. Ten ml conc. H_2SO_4 and 2 g digestion mixture was added to it. The flasks were placed on digestion bench and heated till the solution became clear blue. The flasks were removed, cooled and volume was made to 100 ml with distilled water.

b) Distillation : Aliquot (10 ml) was transferred to micro-kjeldahl assembly and 10 ml of 40% NaOH was added to it. Ten ml of N/100 H_2SO_4 was taken in a conical flask and 2-3 drops of

methyl red indicator was added to it. This conical flask was set under condenser. The distillation was carried for 10-15 min till the red indicator turned blue.

c) **Titration :** The conical flask was removed after washing the tip of condenser with distilled water into the flask. The content of the flask was titrated with N/100 NaOH till the end point reached (red to pink). The volume of alkali used for neutralization of H_2SO_4 was recorded. The amount of nitrogen and hence protein in the sample was calculated using following relationship.

1 ml N/100 H_2SO_4 = 0.00014 g N

$$\% \text{ Crude protein} = \frac{V \times 0.00014 \times D \times 100 \times 5.7}{W \times A}$$

Where :

V = Volume of N/100 H_2SO_4 taken - volume of N/100 NaOH used for titration

D = Dilution factor (volume made in volumetric flask)

D) Enzymes of starch synthesis :

Preparation of developing grains extract :

Ears with developing grains at a stage of 14, 21, 28 and 35 days after anthesis (DAA) were used for preparation of grain extract. The extraction buffer employed was having composition as follows :

Extraction buffer :

50 mM	3-(N-morpholino) propane sulphonic acid (MOPS) pH 7.4
2 mM	$MgCl_2$
1 mM	EDTA and
2 mM	Dithiothritol (DTT)

Nearly 15-20 developing grains amounting to 0.5 g of the grains were removed from middle portion of earheads and were hand homogenized in a prechilled pestle and mortar at 4^0C with cold 2 ml of buffer on ice. The homogenate so obtained was centrifuged at 10,000 xg for 10 min in a refrigerated centrifuge at 4^0C. The supernatant was used as grain extract for enzyme analysis.

Estimation of protein content :

Protein content in the grain extract was estimated using Bradford's dye binding method. For preparing 200 ml of dye, 100 mg of Comassive Brilliant Blue G 250 was dissolved in 50 ml of 95% ethanol and 100 ml of orthophosphoric acid was added. To make the final volume 200 ml, double distilled water was added to it. The reagent was stored in amber coloured bottle in refrigerator. For protein estimation, to 0.1 ml of grain extract, 0.9 ml MOPS buffer and 4 ml of dilute dye (1:4) were added and absorbance was measured at 595 nm. Five

hundred mg of wheat grains of both the varieties obtained at 14, 21, 28 and 35 DAA were dried at 75^0C for 4 days to get the dry weight.

Assay of enzymes :

ADP glucose pyrophosphorylase : AGPase was assay in the reverse direction by modified method of Kleczkowski *et al.* (1993). The reaction mixture contained.

Reagents	Final concentration	Volume in 1 ml reaction
50 mM MOPS (pH 7.4)	1.06 g/30 ml	300 µl
7.5 µ mole $MgSO_4.7H_2O$	18.45 mg/ml	100 µl
Enzyme extract		
3 µ mole 3-PGA	6.912 mg/ml	100 µl
0.5 µ mole NADP+	3.827 mg/ml	100 µl
0.5 µ mole ADP-glucose	3.166 mg/ml	100 µl
Phosphoglucomutase	2 units	3 µl
Glucose-6-phosphate dehydrogenase	2 units	2 µl

The reaction was started by the addition of 200 µl of sodium pyrophosphate (2.5 µ mole). The pyrophosphorolytic activity of AGPase was assayed spectrophotometrically by monitoring the increase in absorbance due to conversion of NADP to NADPH at 340 nm. The data on activity of AGPase was analysed for each of the variety at 15 and 21 DAA and compared with regard to yield potential and the level of high temperature tolerance.

Invertase (EC 3.2.1.26) :

It was assayed by the method of Leigh *et al.* (1979). The reaction mixture (1 ml) contained : phosphate buffer (pH 7.5) 20 mM; sucrose, 25 μ mole and extract. The mixture was incubated at 30^0C for 15 min. The reaction was stopped by adding 1 ml of mixed copper reagent and placed in a boiling water bath for 20 min. Cooled and added 1 ml of arsenomolybdate reagent. The final volume was made to 25 ml and the absorbance read at 520 nm.

Starch branching enzyme (EC 2.4.1.18 α-1,4-glucan-6-glucosyl transferase) :

Decrease in the absorbance of iodine-amylose complex after incubation of enzyme extract with amylose, provided a measure of the activity of starch branching (Q) enzyme (Hawker and Downton, 1974). The reaction mixture (1.5 ml) containing sodium citrate, 100 μ mole; amylose, 300 μg and enzyme extract, 0.1 ml was incubated at 30^0C for 15 min. The reaction was stopped by the addition of 0.5 ml of 2 N HCl followed by the addition of 1 ml of iodine reagent. The iodine reagent was prepared by freshly mixing 0.5 ml of iodine stock solution (6 g KI and 600 mg I_2 in 100 ml of water) to 50 ml of 0.05 N HCl. Water was added to the reaction mixture to give a final volume of 5 ml. The absorbance of the amylose-iodine complex was read at 590 nm and compared to a suitable control.

Starch synthase (EC 2.4.1.21 UDPG (ADPG) : α-1, 4-glucan α-4-glucosyl transferase) :

ADP-glucose and UDP-glucose starch synthase were assayed following the colorimetric method of Leloir *et al.* (1961), using amylopectin as the primer. Reaction mixture (0.4 ml) contained : glycine buffer (pH 8.3), 20 μ mol; EDTA, 0.15 μ mol; amylopectin, 4 mg; glutathione, 1.5 mg and ADP-glucose (or UDP-glucose), 0.5 μ mol. It was incubated with 0.1 ml of enzyme preparation in shaking water bath at 37^0C for 1 h. Reaction was stopped by dipping the reaction mixture in a boiling water bath for 2 min. The ADP (or UDP) formed was measured by the pyruvate kinase procedure. After the reaction was stopped, 20 μl each of phosphoenolpyruvate (0.02 M in 0.4 M KCl) and pyruvate kinase (25 U, freshly diluted with 0.1 M $MgSO_4$) were added and the contents incubated for another 30 min at 37^0C followed by the addition of 0.2 ml of 2, 4-dinitrophenyl hydrazine (0.1% in 2 N HCl), 0.2 ml of 10 N NaOH and 4 ml of 95% ethanol, at an interval of 5 min each. The contents were mixed and centrifuged. The absorbance of the brown coloured supernatant was measured at 520 nm and compared with suitable control in which the reaction mixture was boiled immediately after the addition of enzyme extract.

Sucrose synthase (EC 2.4.2.13) :

It was assayed by the method of Shannon and Dougherty (1972). 0.4 ml of reaction mixture containing UDP, 1 μ mol; sucrose, 20 μ mol and MES buffer (pH 6.5), 60 μ mol was incubated with 0.1 ml of the enzyme preparation at 30^{0}C for 15 min. The reaction was terminated by placing the reaction mixture in a boiling water bath for 2 min. Released fructose was measured by Somogyi's modified method (Somogyi, 1945).

Reagents :

Copper and arsenomolybdate reagents were prepared as already mentioned in the estimation of reducing sugars.

Procedure :

One ml of test extract was taken in a blood sugar tube graduated at 25 ml. Mixed copper reagent (1 ml) was added and then heated for 20 min in a boiling water bath. One ml of arsenomolybdate reagent was added, mixed thoroughly and diluted to 25 ml. A stable blue colour quickly appeared which was read at 520 nm against a suitable blank.

E) Metabolites :

The metabolites were estimated immediately after the samples were harvested. Any change in the physiological state of the tissue may

cause changes in the levels of metabolites. Hence, frozen grains were allowed to drop directly into chilled $HClO_4$.

Extraction :

The metabolites were extracted by the method of Rasi–Caldogno and De–Michelis (1978) with minor modifications. The grains were homogenized in 5 ml of ice-cold 0.8 N $HClO_4$. The homogenate was centrifuged at 10,000 xg for 20 min. The supernatant after neutralization with dropwise addition of 5 M K_2CO_3, was centrifuged again at 7,000 x g for 15 min. The resulting supernatant was made to 10 ml with 0.05 M tris-Cl buffer (pH 7.6). During all subsequent work, the extract was maintained at 0^0C.

Glucose-6-phosphate, glucose-1-phosphate and fructose-6-phosphate :

Estimation of these metabolites was done by a slightly modified method of Latzko and Gibbs (1969). The assay mixture (1.2 ml) contained buffered extract, Tris-Cl buffer (pH 7.5), 60 μ mole; $MgSO_4$, 10 μ mole; NADP, 1 μ mole. The absorbance (E_1) at 340 nm was recorded. G-6-PDH (2U, 10 μl) was added and absorbance (E_2) after the completion of the reaction was noted. E_3 and E_4 were then recorded after the addition of phosphoglucoisomerase (2U, 10μl) and

phosphoglucomutase (2U, 10 μl) respectively. The concentration of G-6-P was calculated by the relation.

$\Delta E_{G\text{-}6\text{-}P} = E_2 - E_1$

$\Delta E_{F\text{-}6\text{-}P} = E_3 - E_2$ gave an estimation of F-6-P.

G-1-P was estimated from the increase in absorbance after the addition of phosphoglucomutase.

UDP-glucose and ADP-glucose :

Estimation of UDPG and ADPG was done by the method of Keppler and Decker (1974). Assay mixture contained : 60 μ mole, Tris-Cl buffer (pH 7.8); 10 μ mole, $MgSO_4$; 1 μ mole, NADP; 0.5 ml metabolite extract; 3 μ mole, sodium pyrophosphate; 2U, glucose-6-phosphate dehydrogenase/phosphoglucomutase. The absorbance (E_1) at 340 nm was recorded. Two units of UDPG pyrophosphorylase was then added and the final absorbance (E_2) recorded. ADPG pyrophosphorylase (2 U) was then added and absorbance (E_3) after the completion of the reaction was noted.

$\Delta E_{UDPG} = E_2 - E_1$ gave an estimation of UDPG.

ADPG was estimated from $\Delta E_{ADPG} = E_3 - E_2$.

Inorganic pyrophosphate (PPi) :

The method of Edwards *et al.* (1984) was followed for PPi estimation. Purified PFP was used to measure the PPi. The F-1,6-P_2 produced by the fructose-2,6-bisphosphatase was measured by coupling to NADH oxidation via aldolase, triose phosphate isomerase and glycerol-3-phosphate dehydrogenase. The reaction mixture in a final volume of 1.2 ml contained : 50 mM HEPES buffer (pH 7.5), 0.3 mM NADH, 5 mM F-6-P, 6mM $MgCl_2$, 1 IU triose phosphate isomerase, 1 U glycerol phosphate dehydrogenase, metabolite extract. The reaction was started by adding purified PFP extract.

CHAPTER-IV

Results

The present investigation was aimed to study the 'Starch metabolism and end use quality of durum wheat in response to high temperature stress'. Two durum wheat varieties WH 896 and WH 912 were screened for their response to high temperature stress. High temperature treatment was given by shifting the pots of both cvs. to polyhouse after heading. The observations recorded during the present investigation were as follows :

Physiological Parameters : Following observations were recorded in flag leaf.

1. Chlorophyll and chlorophyll stability index
2. Chlorophyll fluorescence
3. Membrane thermostability
4. Osmotic potential
5. Dry matter accumulation in grain

Enzymes : These were recorded in developing grains.

1. Invertase
2. Sucrose synthase
3. ADP-glucose pyrophosphorylase

4. Soluble starch synthase

5. Starch branching enzyme

Metabolites : Following observations were recorded in developing grains.

1. Glucose-1-phosphate

2. Glucose-6-phosphate

3. Fructose-6-phosphate

4. ADP-glucose

5. UDP-glucose

6. Inorganic pyrophosphate (PPi)

Biochemical attributes : These were recorded in developing grains.

1. Total soluble sugars

2. Reducing sugars

3. Non-reducing sugars

4. Sucrose

5. Starch

6. Amylose

7. Amylopectin

Quality characteristics : Following observations were recorded in mature grains.

1. Grain shriveling score

2. Grain hardness

3. Grain weight/spike

4. Grain number

5. Test weight (1000-grain weight)

6. β-carotene content

7. Sedimentation value

8. Total protein

9. Starch

The data obtained are presented below :

PHYSIOLOGICAL PARAMETERS

Chlorophyll and chlorophyll stability index (%) :

Results presented in Table 2 and 4 show that total chlorophyll was affected by high temperature stress. Decline in chlorophyll stability index was noted in both the varieties at different days after anthesis under high temperature stress treatment, but decrease as compared to their respective control was higher in WH 912 (26.8%) relative to WH 896 (17.6%).

Chlorophyll fluorescence :

The effect of high temperature on chlorophyll fluorescence at different DAA in shown in Table 3. In WH 896 Fv/Fm decreased 3.6% while in WH 912 it decreased 2.2% under high temperature stress as compared to the control.

Membrane thermostability index (%) :

Data on the performance of two durum wheat varieties at different DAA indicated decrease in the membrane thermostability under high temperature treatment. The decline due to high

temperature stress as compared to their respective control was more in WH 896 (19.2%) as compared to WH 912 (13.5%) (Table 5).

Table 2 : Effect of high temperature on flag leaf total chlorophyll (μ mole g^{-1}) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	70.4±1.7	59.5±0.6	-15.4	65.2±1.3	55.1±0.7	-15.4
21	76.8±1.9	63.0±1.5	-17.9	73.5±1.7	60.2±0.6	-18.0
28	78.4±2.0	62.0±1.5	-20.9	64.1±1.6	50.0±1.0	-21.9
35	66.2±1.8	51.3±2.0	-22.5	51.3±2.0	37.0±1.0	-27.8
Mean	72.9	58.9	-19.2	63.5	50.5	-20.4

Each value is the mean of six replications ± SE

Table 3 : Effect of high temperature on flag leaf chlorophyll fluorescence in durum wheat at different days after anthesis

Days after anthesis	WH 896						% change over the control	WH 912						% change over the control
	Control			High temperature				Control			High temperature			
	F_0	Fm	Fv/Fm	F_0	Fm	Fv/Fm		F_0	Fm	Fv/Fm	F_0	Fm	Fv/Fm	
14	137± 4.56	636± 15.90	0.78± 0.02	114± 6.81	477± 11.73	0.75± 0.02	-3.3	107± 5.90	605± 15.32	0.79± 0.03	126± 7.01	528± 14.51	0.78± 0.03	-0.7
21	143± 4.70	625± 15.73	0.77± 0.02	119± 6.79	475± 11.73	0.74± 0.01	-3.3	132± 7.02	557± 14.68	0.76± 0.02	138± 4.56	514± 14.72	0.72± 0.01	-4.3
28	125± 4.52	552± 14.65	0.76± 0.03	140± 4.69	548± 14.65	0.73± 0.02	-4.0	154± 4.75	578± 14.95	0.72± 0.01	150± 4.74	538± 14.61	0.71± 0.01	-1.7
			0.77			0.74	-3.6			0.76			0.74	-2.2

Each value is the mean of six replications ± SE

Table 4 : Effect of high temperature on flag leaf chlorophyll stability index (%) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	32.1±0.7	25.3±0.5	-21.1	28.4±0.5	22.0±1.0	-22.5
21	28.9±0.5	23.1±1.4	-20.0	25.2±0.5	19.1±0.2	-24.2
28	24.8±0.5	20.2±0.7	-18.5	21.3±0.6	16.2±0.3	-23.9
35	19.6±0.7	18.2±0.5	-7.1	18.4±0.5	11.0±0.3	-40.2
Mean	26.3	21.7	-17.6	23.3	17.0	-26.8

Each value is the mean of six replications ± SE

Table 5 : Effect of high temperature on flag leaf membrane thermastability index (%) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	61.7±3.65	49.9±0.76	-19.1	53.9±3.85	44.3±4.05	-17.8
21	58.2±0.63	52.3±3.05	-10.1	60.0±1.95	51.9±3.05	-13.5
28	57.7±3.05	43.1±4.50	-25.3	50.2±1.05	45.8±0.80	-8.7
35	54.6±0.05	42.2±3.98	-22.7	44.6±2.89	38.4±1.06	-13.9
Mean	58.0	46.8	-19.2	52.1	45.1	-13.5

Each value is the mean of six replications ± SE

Osmotic potential (-MPa) :

Result presented in Table 6 show that flag leaf osmotic potential decreased with high temperature stress in both the varieties at 14 DAA but later on it gradually increased upto 35 DAA in both WH 896 (9.8%) and WH 912 (5.8%) as compared to their respective control.

Dry matter accumulation in grains (mg g^{-1}) :

Reduction in dry matter accumulation in grains was more 15.0% in WH 896 due to high temperature stress relative to control (Table 7). But in WH 912 there was decline in dry matter accumulation in grains at 14 and 21 DAA i.e. 17.5% and 3.8%, respectively and after that it gradually increased at 35 DAA by high temperature treatment compared to unstressed control.

ENZYMES

Acid invertase :

Results presented in Table 8 show that acid invertase activity was higher in WH 896 than WH 912. High temperature treatment led to decrease in acid invertase activity from 14 to 35 DAA in both the varieties. In WH 896, acid invertase activity decreased 1.2% while in WH 912, it decreased 4.7% under high temperature treatment, with respect to unstressed control.

Table 6 : Effect of high temperature on flag leaf osmotic potential (-MPa) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	1.16±0.04	1.57±0.06	-35.3	1.77±0.03	1.56±0.06	-11.8
21	1.74±0.05	1.79±0.03	+2.8	2.00±0.07	1.89±0.07	-5.5
28	1.87±0.06	2.03±0.07	+8.5	2.29±0.08	2.35±0.09	+2.6
35	1.72±0.05	1.74±0.05	+1.1	2.66±0.11	2.41±0.10	-9.3
Mean	1.62	1.78	-9.8	2.18	2.05	-5.8

Each value is the mean of six replications ± SE

Table 7 : Effect of high temperature on developing grains dry weight (mg g^{-1})/Relative growth rate (mg day^{-1}) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	346.0±7.05	286.0±4.70	-17.3	342.0±7.01	282.0±4.69	-17.5
21	458.0±3.29 (0.42)	316.0±4.77 (0.13)	-31.0	368.0±5.12 (0.09)	354.0±7.08 (0.29)	-3.8
28	510.0±3.54 (0.13)	474.0±3.31 (0.59)	-7.0	436.0±2.71 (0.23)	484.0±3.43 (0.46)	+11.0
35	562.0±9.05 (0.13)	518.0±3.55 (0.13)	-7.8	376.0±5.34 (0.13)	546.0±3.10 (0.16)	+14.7
Mean	469.0	398.5	-15.0	405.5	416.5	+2.7

Each value is the mean of six replications ± SE
* Values of RGR shown in parentheses

Table 8 : Effect of high temperature on developing grains acid invertase activity (nmole min^{-1} mg $protein^{-1}$) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	8.3±0.96	8.0±0.95	-3.6	7.4±0.33	6.3±0.27	-14.8
21	9.7±0.38	7.7±0.36	-21.7	7.0±0.23	2.8±0.09	-60.0
28	6.3±0.27	5.4±0.09	-14.2	3.2±0.18	ND	
35	4.2±0.12	ND		1.5±0.05	ND	
Mean	7.1	7.0	-1.2	4.7	4.5	-4.7

Each value is the mean of six replications ± SE

ND – Not detectable

Table 9 : Effect of high temperature on developing grains sucrose synthase activity (nmole min^{-1} mg $protein^{-1}$) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	22.2±1.0	19.5±0.2	-12.1	24.0±0.4	18.2±0.5	-24.1
21	24.1±0.4	22.0±1.0	-8.7	21.3±0.6	15.5±0.5	-27.2
28	25.7±0.5	20.1±0.7	-21.7	18.6±0.5	13.0±0.4	-30.1
35	20.8±0.8	17.3±0.6	-16.8	15.2±0.2	11.1±0.3	-26.9
Mean	23.2	19.7	-14.9	19.7	14.4	-26.9

Each value is the mean of six replications ± SE

Table 10 : Effect of high temperature on developing grains ADP glucose pyrophosphorylase activity (nmole min^{-1} mg $protein^{-1}$) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	34.0±2.3	24.1±0.9	-29.1	30.9±2.5	20.4±0.2	-33.9
21	75.9±3.2	46.8±2.1	-38.3	71.8±4.4	43.0±1.9	-40.1
28	78.4±3.6	55.5±0.1	-29.2	70.2±4.3	43.0±1.0	-38.7
35	69.1±2.7	49.2±0.9	-28.7	66.7±4.4	22.9±0.7	-65.6
Mean	64.3	43.9	-31.7	59.9	32.3	-46.0

Each value is the mean of six replications ± SE

Table 11 : Effect of high temperature on developing grains soluble starch synthase activity (nmole min^{-1} mg $protein^{-1}$) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	0.8±0.04	0.5±0.02	-37.5	0.7±0.03	0.4±0.11	-42.8
21	2.7±0.12	2.3±0.06	-14.8	1.8±0.07	0.9±0.04	-50.0
28	3.9±0.16	2.1±0.04	-46.1	2.4±0.06	1.7±0.06	-29.1
35	3.2±0.13	1.3±0.05	-59.3	1.9±0.07	0.9±0.04	-52.6
Mean	2.6	1.5	-41.5	1.7	0.9	-42.6

Each value is the mean of six replications ± SE

Sucrose synthase :

WH 896 had higher mean sucrose synthase activity relative to WH 912 (Table 9). Sucrose synthase activity increased drastically at 21 DAA in WH 896 (22.0 n mole min^{-1} mg $protein^{-1}$) and then decreased upto 35 DAA. However, in WH 912, gradual decrease was observed upto 35 DAA. High temperature treatment caused 14.9% reduction in WH 896 and 26.9% in WH 912 in sucrose synthase activity as compared to their respective control.

ADP-glucose pyrophosphorylase :

Average AGPase activity was lower in WH 912 related to WH 896 (Table 10). AGPase activity increased drastically upto 28 DAA in both the varieties then decreased at 35 DAA. In WH 912, AGPase activity reduced more 46.0% than WH 896 (31.7%) under high temperature treatment compared to untreated control.

Soluble starch synthase :

Data on the activity of soluble starch synthase in both the varieties are given in Table 11. Soluble starch synthase activity increased drastically at 21 DAA in WH 896 (2.3 n mole min^{-1} mg $protein^{-1}$) and then decreased to 1.3 n mole min^{-1} mg $protein^{-1}$ at 35 DAA. However, in WH 912, gradual increase in soluble starch synthase activity was observed upto 28 DAA. Higher decline 42.6% in SSS

activity was noted in WH 912 as compared to WH 896 (41.5%) under high temperature treatment with respect to unstressed control.

Table 12 : Effect of high temperature on developing grains starch branching enzyme (nmole min^{-1} mg protein^{-1}) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	8.0±0.37	6.8±0.21	-15.0	6.4±0.14	3.5±0.14	-45.3
21	6.2±0.14	5.9±0.18	-4.8	5.8±0.17	3.1±0.12	-46.5
28	8.5±0.41	5.3±0.14	-37.6	4.9±0.20	2.8±0.06	-42.8
35	4.9±0.22	3.6±0.08	-26.5	3.0±0.68	2.2±0.05	-26.6
Mean	6.9	5.4	-21.7	5.0	2.9	-42.2

Each value is the mean of six replications ± SE

Table 13 : Effect of high temperature on developing grains levels of glucose-6-phosphate (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	16.5±0.3	13.0±0.4	-21.2	14.1±0.4	11.0±0.4	-22.0
21	25.5±0.3	24.9±1.7	-2.3	28.7±1.2	23.5±1.5	-18.2
28	21.0±1.4	18.0±1.0	-14.0	19.5±0.3	16.0±0.4	-17.7
35	18.0±1.0	15.2±0.2	-15.5	18.4±1.1	12.7±0.5	-31.0
Mean	20.2	17.8	-12.1	20.2	15.8	-21.6

Each value is the mean of six replications ± SE

Table 14 : Effect of high temperature on developing grains levels of glucose-1-phosphate (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	0.6±0.04	0.5±0.02	-23.0	0.5±0.03	0.4±0.01	-23.6
21	1.1±0.01	1.0±0.03	-9.0	1.1±0.07	0.9±0.06	-18.2
28	0.8±0.03	0.7±0.02	-15.6	0.7±0.03	0.6±0.05	-19.2
35	0.7±0.01	0.6±0.04	-16.6	0.7±0.04	0.5±0.02	-32.0
Mean	0.8	0.7	-15.1	0.8	0.6	-22.6

Each value is the mean of six replications ± SE

Starch branching enzyme :

Higher starch branching enzyme activity was noted in WH 896 as compared to WH 912 (Table 12). In both the varieties, branching enzyme activity decreased from 14 to 35 DAA under high temperature treatment but the activity was less reduced in WH 896 (21.7%) than in WH 912 (42.2%), as compared to the control.

METABOLITES

Glucose-6-phosphate :

Results presented in Table 13 show that glucose-6-phosphate declined in both the varieties under high temperature treatment. The level of G-6-P increased drastically at 21 DAA and then decreased upto 35 DAA in both the varieties. The reduction was 12.1% in WH 896 and 21.6% in WH 912 in comparison to control.

Glucose-1-phosphate :

Data on glucose-1-phosphate in both varieties under high temperature treatment shown in Table 14. The level increased upto 21 DAA and then decline in both the varieties. The magnitude of decline 15.1% and 22.6% in WH 896 and WH 912, respectively observed as compared to unstressed control.

Table 15 : Effect of high temperature on developing grains levels of fructose-6-phosphate (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	3.5±0.1	2.7±0.1	-21.4	3.0±0.1	2.2±0.1	-25.6
21	5.7±0.2	5.2±0.2	-7.8	5.7±0.2	4.7±0.2	-18.2
28	4.2±0.3	3.6±0.2	-13.3	4.0±0.3	3.2±0.1	-20.0
35	3.6±0.2	3.0±0.1	-16.6	3.8±0.2	2.5±0.2	-33.2
Mean	4.2	3.6	-13.8	4.1	3.1	-23.4

Each value is the mean of six replications ± SE

Table 16 : Effect of high temperature on developing grains levels of ADP-Glucose (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	0.7±0.04	0.4±0.02	-38.8	0.4±0.01	0.3±0.00	-31.1
21	2.8±0.05	1.5±0.11	-47.1	1.6±0.10	1.0±0.06	-37.4
28	4.0±0.03	2.9±0.09	-25.9	2.8±0.08	2.1±0.06	-26.3
35	2.0±0.00	0.7±0.05	-63.5	0.7±0.21	0.5±0.23	-21.4
Mean	2.3	1.4	-41.0	1.4	1.0	-29.3

Each value is the mean of six replications ± SE

Table 17 : Effect of high temperature on developing grains levels of UDP-glucose (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	2.2±0.11	1.3±0.06	-40.0	1.4±0.08	0.9±0.04	-37.1
21	8.5±0.44	4.6±0.14	-45.8	4.9±0.21	3.0±0.06	-38.7
28	12.0±0.43	8.8±0.46	-26.5	8.7±0.53	6.4±0.21	-26.0
35	6.0±0.22	2.2±0.11	-63.4	1.9±1.12	1.6±0.05	-14.5
Mean	7.1	4.2	-41.0	4.2	3.0	-29.3

Each value is the mean of six replications ± SE

Table 18 : Effect of high temperature on developing grains levels of inorganic pyrophosphate (nmole g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	0.2±0.03	0.1±0.02	-28.0	0.2±0.02	0.1±0.03	-45.0
21	0.5±0.01	0.4±0.01	-20.3	0.4±0.05	0.2±0.06	-40.4
28	0.4±0.03	0.3±0.01	-35.4	0.3±0.02	0.1±0.05	-51.4
35	0.2±0.00	0.1±0.01	-44.4	0.1±0.07	0.1±0.04	-52.6
Mean	0.3	0.2	-30.5	0.3	0.1	-46.2

Each value is the mean of six replications ± SE

Fructose-6-phosphate :

The data presented in Table 15 showed level of fructose-6-phosphate in WH 896 and WH 912. In both the varieties, level increased upto 21 DAA and then decline. In WH 896, it decreased less 13.8% as compared to WH 912 (23.4%) under high temperature treatment.

ADP-glucose :

Data on ADP-glucose in both the varieties are shown in Table 16. In both the varieties, level of ADP-glucose increased upto 28 DAA and then decline. After high temperature treatment, the reductions were 29.3% and 41.0% in WH 912 and WH 896, respectively as compared to unstressed control.

UDP-glucose :

Higher UDP-glucose was noted in WH 912 than WH 896 (Table 17). High temperature treatment led to increase in UDP-glucose upto 28 DAA in both the varieties. In WH 896 it decreased to 41.0%, while in WH 912, it reduced to 29.3% under high temperature treatment, with respect to unstressed control.

Inorganic pyrophosphate (PPi) :

Lower inorganic pyrophosphate was observed in WH 912 relative to WH 896 (Table 18). In both varieties, the level of PPi was gradually

Table 19 : Effect of high temperature on developing grains total soluble sugars (mg g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	121.3±3.1	108.6±3.0	-10.4	115.1±3.0	97.7±2.4	-15.1
21	70.5±1.7	53.0±1.5	-24.8	62.7±1.9	39.0±1.1	-37.7
28	52.4±1.5	34.2±1.1	-34.7	38.3±1.0	16.2±1.0	-57.7
35	31.0±0.9	22.8±0.9	-26.4	24.0±1.1	11.4±0.9	-52.5
Mean	68.8	54.6	-20.5	60.0	41.0	-31.5

Each value is the mean of six replications ± SE

Table 20 : Effect of high temperature on developing grains reducing sugar (mg g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	**High temperature**	**% change over the control**	**Control**	**High temperature**	**% change over the control**
14	35.4±1.0	24.5±1.1	-30.7	28.8±1.2	21.0±1.0	-27.0
21	18.2±0.9	14.3±1.0	-21.4	16.6±0.9	11.0±0.9	-33.7
28	15.0±1.1	9.2±0.9	-38.6	10.7±0.9	6.3±0.4	-41.1
35	8.1±0.5	6.0±0.4	-25.9	7.9±0.3	4.1±0.3	-48.1
Mean	19.1	13.5	-29.5	16.0	10.6	-33.7

Each value is the mean of six replications ± SE

Table 21 : Effect of high temperature on developing grains non-reducing sugar (mg g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	85.9±2.3	84.1±2.3	-2.0	86.3±2.4	76.7±1.9	-11.1
21	52.3±1.1	38.7±1.1	-26.0	46.1±3.1	28.0±1.1	-39.2
28	37.4±1.1	25.0±1.1	-33.1	27.6±1.1	9.9±0.9	-64.1
35	22.9±0.8	16.8±0.9	-26.6	16.1±1.1	7.3±0.4	-54.6
Mean	49.6	41.4	-17.0	44.0	30.4	-30.7

Each value is the mean of six replications ± SE

increased upto 21 DAA and then decline at 28-35 DAA. High temperature treatment caused 46.2% decline in WH 912 while in WH 896 it declined to 30.5% as compared to control.

BIOCHEMICAL ATTRIBUTES

Total soluble sugars (mg g^{-1} dry weight) :

Lower total soluble sugars was noted in WH 912 relative to WH 896 at different DAA under high temperature treatment (Table 19). High temperature treatment led to decrease of 31.5% in WH 912 and of 20.5% in WH 896.

Reducing sugars (mg g^{-1} dry weight) :

The result on reducing sugars content is presented in Table 20. At 14 DAA reduction in reducing sugar content was more in WH 896 (30.7%) as compared to WH 912 (27.0%). But after that reduction were more in WH 912 (33.7%) than in WH 896 (29.5%) due to high temperature treatment as compared to the control.

Non-reducing sugars (mg g^{-1} dry weight) :

Grain non-reducing sugars of WH 896 and WH 912 are shown in Table 21. The non-reducing sugars decreased continously from 14 to 35 DAA in both varieties. The decline was to the magnitude of 17.0%

and 30.7% in WH 896 and WH 912, respectively as compared to unstressed control.

Table 22 : Effect of high temperature on developing grains sucrose (mg g^{-1} dry wt) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	50.3±2.0	45.1±1.1	-10.3	45.8±1.1	38.0±0.9	-17.0
21	31.6±1.5	28.7±0.9	-9.1	29.1±0.9	18.7±1.0	-35.7
28	16.2±1.0	13.4±0.7	-17.2	13.9±0.7	9.8±0.5	-29.4
35	14.9±1.0	9.0±0.5	-39.5	11.5±0.8	7.6±0.4	-33.9
Mean	28.2	24.0	-14.8	25.0	18.5	-26.1

Each value is the mean of six replications ± SE

Table 23 : Effect of high temperature on developing grains starch (mg g^{-1} dry wt.) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	422.1±2.7	316.0±4.7	-25.1	397.0±5.1	273.1±4.6	-31.2
21	486.0±4.0	364.2±5.1	-25.0	443.2±2.7	301.4±4.3	-31.9
28	564.3±8.1	406.0±5.1	-28.0	501.5±4.0	332.0±4.7	-33.7
35	607.2±7.3	430.7±2.7	-29.0	536.7±5.3	354.8±5.0	-33.8
Mean	519.9	379.2	-27.0	469.6	315.3	-32.8

Each value is the mean of six replications ± SE

Table 24 : Effect of high temperature on developing grains amylose (mg g^{-1} dry wt) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	105.1±2.1	120.0±1.9	+14.1	100.0±2.1	109.5±3.6	+9.5
21	121.2±3.0	145.0±1.9	+19.6	110.0±3.0	121.9±3.6	+10.8
28	141.1±3.8	150.1±2.1	+6.3	125.1±3.1	140.0±2.3	+11.9
35	151.7±3.8	172.0±2.0	+13.3	136.6±3.1	150.5±2.4	+10.1
Mean	129.7	146.7	+13.0	117.9	130.4	+10.6

Each value is the mean of six replications ± SE

Table 25 : Effect of high temperature on developing grains amylopectin (mg g^{-1} dry wt) in durum wheat at different days after anthesis

Days after anthesis	WH 896			WH 912		
	Control	High temperature	% change over the control	Control	High temperature	% change over the control
14	317.0±4.7	196.0±3.0	-38.1	297.0±4.1	163.6±2.1	-44.9
21	364.8±5.1	219.2±4.7	-39.9	333.2±3.7	179.5±2.5	-46.1
28	423.2±2.7	255.9±4.0	-39.5	376.4±4.6	192.0±3.0	-48.9
35	455.5±3.5	258.7±4.8	-43.2	400.1±2.8	204.3±4.4	-48.9
Mean	390.1	232.1	-40.4	351.6	184.8	-47.4

Each value is the mean of six replications ± SE

Sucrose (mg g^{-1} dry weight) :

Sucrose decreased drastically from 14 to 35 DAA in grains of both the varieties under high temperature treatment when compared with the control (Table 22). Decrease in sucrose was higher in WH 912 (26.1%) than WH 896 (14.8%).

Starch (mg g^{-1} dry weight) :

Results presented in Table 23 revealed that starch was more in WH 896 relative to WH 912. A gradual decline in starch was observed with progress in grain development (14 to 35 DAA). High temperature treatment caused 27.0% reduction in grain starch in WH 896 than WH 912 (32.8%) as compared to the control.

Amylose (mg g^{-1} dry weight) :

Higher amylose was noted in WH 896 as compared to WH 912 (Table 24). In both the varieties, a sharp increase in amylose was observed at different DAA under high temperature treatment but increase in WH 912 was less 10.6% than WH 896 (13.0%), when compared with their respective control.

Amylopectin (mg g^{-1} dry weight) :

The two varieties had differential response to the high temperature stress. The reduction in amylopectin from 14 to 35 DAA

due to high temperature was 47.4% in WH 912 and 40.4% in WH 896 (Table 25).

Table 26 : Effect of high temperature on various grain quality characteristics in durum wheat

	WH 896			WH 912		
	Control	**High temperature**	**% change over the control**	**Control**	**High temperature**	**% change over the control**
Grain shriveling score (%)	24.3±0.4	38.3±1.0	+57.6	26.0±0.5	46.3±2.1	+78.0
Grain hardness (kg/seed)	16.9±0.3	13.7±0.2	-18.9	16.7±0.3	9.7±0.1	-41.9
Grain wt. 5 $Spike^{-1}$ (gram)	12.1±0.1	11.3±0.1	-6.6	11.1±0.1	9.2±0.1	-17.1
Grain number 5 $Spike^{-1}$	275±3.5	271±9.0	-1.4	271±9.0	256±3.4	-5.5
Test weight [1000-grain weight (g)]	46±3.1	41.6±4.5	-9.5	40.9±4.2	35.9±2.5	-12.2

Each value is the mean of six replications ± SE

Table 27 : Effect of high temperature on various grain quality characteristics in durum wheat

	WH 896			WH 912		
	Control	**High temperature**	**% change over the control**	**Control**	**High temperature**	**% change over the control**
β-carotene content (μg g^{-1})	6.0±0.1	4.9±0.2	-18.3	6.8±0.1	4.1±0.2	-39.7
Sedimentation value (ml)	35±1.0	37±0.7	+5.7	30±1.0	25±1.1	-16.6
Total protein (mg g^{-1})	137±3.0	117±2.0	-17.0	129±2.9	125±2.3	-3.1
Starch (mg g^{-1})	608±9.4	460±8.8	-24.3	547±9.3	375±8.5	-31.4

Each value is the mean of six replications ± SE

GRAIN QUALITY CHARACTERISTICS

Grain shriveling score (%) :

Results presented in Table 26 show that in both the varieties, grain shriveling score increased due to high temperature stress treatment. In WH 896, it increased less 57.6% in relation to WH 912 (78.0%) under high temperature treatment with respect to their control.

Grain hardness (kg/seed) :

In both the varieties, grain hardness decreased due to high temperature treatment (Table 26). But in WH 896, it declined less 18.9% as compared to WH 912 (41.9%) under high temperature stress.

Grain weight 5 spike^{-1} (g) :

Data related to grain weight 5 spike^{-1} presented in Table 26 revealed that high temperature treatment caused 6.6% and 17.1% reduction in WH 896 and WH 912, respectively with respect to unstressed plants.

Grain number 5 spike^{-1} :

It was evident from the data (Table 26) that higher number of grain 5 spike^{-1} were obtained in WH 896 relative to WH 912. After high

temperature treatment, the reduction was more in WH 912 (5.5%) and less in WH 896 (1.4%) with respect to their control.

Test weight (g) :

It was observed that 1000-grain weight was comparatively higher in WH 896 than WH 912 (Table 26). After high temperature treatment, the reduction was 9.5% and 12.2% in WH 896 and WH 912 respectively, compared to unstressed plants.

β-carotene (μg g^{-1}) :

In both the varieties, β-carotene content declined due to high temperature treatment (Table 27). In WH 896, it declined less 18.3% relative to WH 912 (47.2%) under high temperature treatment with respect to their control.

Sedimentation value (ml) :

Both the varieties differed in their sedimentation value in response to high temperature treatment (Table 27). In WH 896, sedimentation value increased 5.7% while in WH 912, it decreased 16.6% under high temperature treatment with respect to unstressed control.

Total protein (mg g^{-1}) :

WH 896 had higher mean protein content than the WH 912 (Table 27). High temperature treatment caused 3.1% reduction in WH 912 and 22.3% in WH 896 as compared to their control.

Starch (mg g^{-1}) :

Higher starch content was noted in WH 896 relative to WH 912 (Table 27). High temperature treatment led to reduction in starch in both the varieties. But the magnitude of reduction was higher in WH 912 (31.4%) as compared WH 896 (24.3%) with respect to unstressed plants.

CHAPTER-V

Discussion

Wheat, a thermo-sensitive, long day crop is the most extensively cultivated crop in the world with latitudinal distribution from 30-60^{0}N and 27-40^{0}S. The crop is best adapted to the temperate climate. Temperature higher than optimum is known to affect the various stages of growth and productivity in cereals (Maestri *et al.*, 2002). It has been shown in crops like wheat that high temperature during the grain filling period has affected both grain quality and yield (Kumar *et al.*, 2004). Achievements made in breeding for temperature tolerance in cereals to date are not very significant. It is mainly because of, use of progenitor germplasm with narrow range of genetic diversity for stress resistance traits, including heat stress tolerance (Holden *et al.*, 1993). A search for beneficial wild alleles as a source of tolerant gene pool is a prerequisite for improving the existing cultivars either by traditional hybridization program or through biotechnological approaches.

High temperature stress (>35^{0}C) during the grain filling period has the potential to modify grain quality. Starch is the main component which constitutes 75% of the wheat kernel and has a direct affect on the nature and quality of flour and end products of wheat. The attempts to increase starch content in cereals have a direct bearing on the food industry. Starch content can be manipulated by chemical means provided we have adequate information on the pathway of starch biosynthesis and the control mechanisms associated with it. The control mechanisms associated with the pathway of starch biosynthesis provide chemical means to manipulate starch content and grain yield.

Therefore, the present investigation was undertaken with a view to : Find out the biochemical and physiological basis of high temperature induced changes in sucrose to starch conversion and determine the response of high temperature stress on grain quality. Studies were carried out on wheat cvs. WH 896 and WH 912. The observations recorded are discussed under the following sections :

I) Physiological parameters :

High temperature brings about a general loss of total chlorophyll in wheat by affecting chlorophyll 'a', chlorophyll 'b' and carotenoids (Shubhra *et al.*, 2006). In the present investigations, data on total chlorophyll content in two durum wheat varieties indicated a reduction

under high temperature conditions as compared to their respective control (Table 2). Physiological evidences indicate that loss of chlorophyll during grain filling is associated with reduced yield in the field (Reynolds *et al.*, 1998).

Chlorophyll fluorescence analysis may provide a sensitive indicator of stress conditions in plants. Fluorescence transient can be used to address the effects of heat on crop productivity. High temperature decreased mean chlorophyll fluorescence by 42 per cent and 11 per cent in seedlings and maturing plants, respectively (AI-Khatib and Paulson, 1990). The present investigation also indicated the loss of chlorophyll fluorescence under high temperature treatment in comparison to their respective control (Table 3). Similarly, the results pertaining to photosynthetic damage due to high temperature were reported by (Abrol and Ingram, 1996; Mohanty and Vani, 2003; Lu and Lu, 2004; Brandner and Salvucci, 2004; Kim and Ports Jr., 2005).

Membrane structure was affected most by temperature. Therefore, membrane thermostability is an important parameter for screening thermotolerance (Blum *et al.*, 2001; Munjal and Dhanda, 2004). In the present investigation, WH 896 showed 19.2% and WH 912 (13.5%) reduced membrane stability under high temperature stress as compared to their respective control (Table 5). Similarly, Taiz and Zeiger

(2002) reported that high temperature stress leads to loss of membrane integrity, followed by increase in cell permeability. This leads to increased ion and solute flux (Levitt, 1980). High temperature induced leaching of inorganic solutes was the result of disruption of membrane structure caused by denaturation of membrane proteins and melting of membrane lipids (Abrol and Ingram, 1996).

Stress-induced decrease of osmotic potential in *Triticum aestivum* has been reported to be mainly due to active accumulation of sugars (Munns and Weir, 1981; Johnson *et al.*, 1984) and amino acids (Munns *et al.*, 1979; Drossopoulos *et al.*, 1985). The high temperature stress and drought reduced osmotic potential in wheat and sorghum (Machado and Paulsen, 2001). The results presented in Table 6 also showed the decrease in osmotic potential in both the varieties under high temperature treatment as compared to their respective control.

Temperature is a major factor affecting the growth and development of wheat during early phase of floret formation, grain set and their development. In the present investigation, the dry matter accumulation was drastically decreased days after anthesis in WH 896 while increased in WH 912 under high temperature stress. The correlations between relative water content (RWC), osmotic potential and both grain yield (GY) and harvest index (HI) were positive (Merah, 2001).

Sohu *et al.* (2002) have reported that heat stress during anthesis to maturity reduces the duration of GS 3 phase resulting into decreased duration and rate of grain filling, reduction in grain number, lower photosynthesis and lower grain weight ultimately leading to poor sink filling.

II) Enzymes :

Results presented in Table 8 revealed that activity of acid invertase was higher in grains of WH 896 than WH 912 at different DAA. Grain acid invertase activity decreased due to high temperature stress by 1.2% in WH 896 and by 4.7% in WH 912 in comparison to their respective control. Decline in invertase activity by high temperature stress in WH 912 may be due to decrease in sucrose breakdown as compared to WH 896. These results were further supported by Flugge and Heldt (1991); Taiz and Zeiger (2002).

There was 40-50% decrease in sucrose synthase activity measured under optimal conditions in extracts from potato tubers exposed to high temperature (Lafta and Lorenzen, 1995). Similarly, data presented in present study indicate that the activity of sucrose synthase was scarcely affected. Similar results were also observed by Jenner (1991) that the mass-action ratio of sucrose synthase reaction was perturbed at high temperature.

The activity of AGPase was reduced by upto 35% in the endosperm of grains heated at 35^{0}C (Hawker and Jenner, 1993). High temperature stress affects starch biosynthesis by decreasing AGPase activity. Data in Table 10 revealed that activity of ADP-glucose pyrophosphorylase was higher in grains of WH 896 as compared to WH

912 at different DAA under high temperature stress. Heat stress affects AGPase more than other enzymes and it may contribute to a reduction in the efficiency of starch synthesis (Singletary *et al.*, 1994).

In the present investigation, there was relatively more decrease in soluble starch synthase activity in WH 912 than in WH 896 under high temperature stress (Table 11). The conversion of sucrose to starch is impaired at high temperature and limits starch synthesis (Bhullar and Jenner, 1985). Among the enzymes, involved in the pathway of sucrose to starch metabolism in wheat endosperms, soluble starch synthase is the one which is most sensitive to high temperature (Hawker and Jenner, 1993; Jenner *et al.*, 1993 and Keeling *et al.*, 1993). The reduction in the rate of grain growth above 30^{0}C is mainly due to the reduced activity of soluble starch synthase (Zahedi *et al.*, 2003).

More reduction in starch branching enzyme activity in WH 912 as compared to that in WH 896 was observed. The activity of starch branching enzyme was not significantly affected by exposure to a temperature of 35^{0}C (Hawker and Jenner, 1993). Jiang *et al.* (2003) reported that the branch chain pattern of amylopectin is changed by temperatures and it is due to reduced activity of branching enzyme at high temperature.

III) Metabolites :

The effect of high temperature on metabolites have been presented in Table 13-18. ADP-glucose is produced from sucrose in the endosperm and is the immediate substrate for starch synthesis. There was no correlation between the changes in sucrose and ADP-glucose concentration at high temperature (Zahedi *et al.*, 2003). Data of present investigation also showed the decrease in the levels of ADP-glucose and UDP-glucose at different DAA under high temperature stress as compared to control.

The amount of ADPG decreased in potato tubers when exposed to elevated temperature (>35^{0}C). The decrease in ADPG correlated with the reduction in starch. There was strong correlation between sucrose phosphate synthase activation and *in vivo* level of G-6-P. As the temperature was increased from 19^{0}C to 37^{0}C, G-6-P level increased progressively. No consistent changes were observed in the levels of ADP-glucose, UDP-glucose or PPi. Jenner (1991) reported that the increased in temperature in wheat decreased the level of metabolites. The overall decrease in metabolite levels in wheat during long-term exposure to heat indicates that there may be additional factors besides the inhibition of starch synthase that contribute to a small increase in starch deposition with increasing temperature (Geigenberger *et al.*, 1998).

IV) Biochemical attributes :

The effect of high temperature on biochemical parameters were presented in Table 19-25. There was reduction in biochemical parameters under high temperature stress in both the varieties. The reduction was more in WH 912 than WH 896 except in case of amylose. The amylose percentage increased under high temperature treatment. At 30^{0}C, amounts of both protein and starch are reduced but the reduction in starch was more than that of protein (Bhullar and Jenner, 1985). In the present study, the reduction in starch was more than that of protein at different DAA under high temperature stress as compared to their respective control. It, therefore, appears that during grain filling at high temperature in addition to reduced synthesis of some starch metabolic enzymes, the catalytic properties of other enzymes are also altered.

Numerous studies involving maize (Jones *et al.*, 1984; Keeling *et al.*, 1994; Singletary *et al.*, 1994), wheat (Bhullar and Jenner, 1985) and barley (MacLeod and Duffus, 1988) have shown a negative effect of heat stress on starch deposition in the kernel. This may be due to impairment of conversion of sucrose to starch at high temperature and limits starch synthesis (Bhullar and Jenner, 1985). There was no correlation between the changes in sucrose and ADPG concentrations at high temperature (Zahedi *et al.*, 2003). The components of starch

were reduced at high temperature (Zahedi, 2001). Decreases in the activities of AGPase has been observed both in maize kernels and wheat endosperms under heat stresses, thus resulting in a reduction of starch synthesis (Keeling *et al.*, 1993; Wilhelm *et al.*, 1999). High temperature exerts its effects by disturbing sucrose : starch balance in wheat (Bhullar and Jenner, 1985). High temperature decreased soluble sugar content in wheat (Shah and Paulson, 2005).

The stability of Granule Bound Starch synthase at moderately high temperature may explain the increase in the percentage of amylose in the grains (Shi *et al.*, 1994; Tester *et al.*, 1995). So, there is progressive increase in the grain amylose percentage over the grain-filling period, especially at high temperature (Zahedi *et al.*, 2004).

V) Grain quality characteristics :

Both grain yield and quality are adversely affected by high temperature stress (Table 26-27). High temperature during grain filling in wheat limits the grain yield (Zahedi *et al.*, 2003). Zhong-Hu and Rajaram (1994) concluded that the grain filling rate was more temperature sensitive than days to anthesis and duration of grain filling. Similarly, high temperature during kernel filling decreased wheat yield by reducing kernel weight (Warrington *et al.*, 1977; Tashiro and Wardlaw, 1990a; Stone and Nicolas, 1994). Elevated temperatures during grain filling decrease starch deposition and therefore, adversely

affect yield (Gibson and Paulsen, 1999). Grain weight seems to be less sensitive to heat stress than grain number (Abrol and Ingram, 1996). Under heat stress conditions, 1000-grains weight had the highest positive direct effect and significant positive correlation with grain yield while days to heading had high negative direct effect and significant negative correlation with grain yield in wheat (Munjal and Dhanda, 2004).

During heat stress, the SDS sedimentation volumes either became higher or remained at similar levels (Bencze *et al.*, 2004). Grain protein accumulation declines as the temperature increases, although it is usually less sensitive than starch. High temperature stress (>35^{0}C) during the grain filling period has the potential to modify grain quality. Total soluble protein is also reduced by high temperature. This might be due to protein denaturation and inhibition of protein synthesis at higher temperature, since the injury from high temperature has often been attributed to the denaturation of proteins (Blumenthal *et al.*, 1995a).

Wheat kernels filled at 40^{0}C showed a wrinkled surface because of shriveling. High temperature soon after fertilization may results in abnormal cell division, which explains the production of shrunken kernels (Tashiro and Wardlaw, 1990a).

Elevated temperature during grain-filling may somehow weaken the starch-protein interaction and thereby decrease kernel hardness (Shi *et al.*, 1994).

Variety WH 896 was found more tolerant to heat stress than WH 912 when they were exposed to same degree of stress.

CHAPTER-VI

Summary and Conclusion

The present investigation was aimed to study the starch metabolism and end use quality of durum wheat in response to high temperature stress. Two durum wheat varieties WH 896 and WH 912 were used to study the response of various physiological processes to high temperature stress. The plants were raised in screen house.

The main observations recorded include physiological parameters (chlorophyll, chlorophyll fluorescence, chlorophyll stability index, membrane thermostability, osmotic potential and dry matter accumulation in grain), activities of enzymes, metabolities, biochemicals involved in starch metabolism and grain quality characteristics. Observations were taken from 14 to 35 days after anthesis (DAA) for physiological and biochemical studies while quality characteristics observations were recorded from harvested grains.

Results show that various physiological, biochemical and grain quality characteristics were adversely affected by high temperature in

durum wheat. Salient findings of this experimentation are enlisted below :

PHYSIOLOGICAL PARAMETERS

- High temperature declined flag leaf chlorophyll and chlorophyll fluorescence in both the varieties. The reduction was 3.6% and 2.2% in WH 896 and WH 912, respectively.
- Chlorophyll stability index (%) declined in both the varieties under high temperature as compared to their respective control. But decrease was more in WH 912 (26.8%) than WH 896 (17.6%).
- The reduction in membrane thermostability index (%) was less in WH 912 (13.5%) as compared to WH 896 (19.2%) over the control.
- Overall dry matter accumulation was higher in WH 896 (469 mg g^{-1}) than WH 912 (416.5 mg g^{-1}) under control condition. But high temperature decreased grains dry weight accumulation in WH 896 (15.0%) while increased in WH 912 (2.7%) as compared to their respective control.
- Flag leaf osmotic potential was reduced more in WH 896 (9.8%) than WH 912 (5.8%) under high temperature treatment.

- Among all the physiological parameters, leaf chlorophyll stability index and membrane thermostability index were maximum sensitive to high temperature tress. The reductions in flag leaf chlorophyll stability index and flag leaf membrane thermostability index were more in WH 912 and WH 896, respectively.

ENZYMES

- Soluble starch synthase activity decreased by high temperature treatment in both the varieties. The reduction was more in WH 912 (42.6%) than WH 896 (41.5%).
- ADP-glucose pyrophosphorylase activity declined more (46.0%) in WH 912 as compared to WH 896 (31.7%) under high temperature treatment.
- Activities of starch branching enzyme, sucrose synthase and acid invertase declined more in WH 912 in relation to WH 896 under high temperature treatment.
- The activity of soluble starch synthase (SSS) was reduced more in WH 896 (41.5%). However, ADP-glucose pyrophosphorylase activity decreased more in WH 912 (46.0%) as compared to the reduction in all other enzyme activities under high temperature treatment.

METABOLITES

- Magnitude of reduction in ADP-glucose and UDP-glucose metabolites were same in WH 896 (41.0%) and WH 912 (29.3%) under high temperature stress as compared to control.
- The level of inorganic pyrophosphate declined more in WH 912 (46.2%) as compared to WH 896 (30.5%) under high temperature treatment.
- Glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate levels decreased more in WH 912 than WH 896 under high temperature treatment.
- Among all the metabolites, the reduction due to high temperature treatment was found more in ADP-glucose, UDP-glucose and inorganic pyrophosphate.

BIOCHEMICAL ATTRIBUTES

- Amylopectin reduced with increasing grain growth in both the varieties. But the reduction was more in WH 912 (47.4%) than WH 896 (40.4%) under high temperature stress.
- The decline in reducing sugars was less in WH 896 (29.5%) as compared to WH 912 (33.7%) under high temperature treatment.

- Starch, total soluble sugars, non-reducing sugars and sucrose decreased more in WH 912 as compared to WH 896 under high temperature treatment but the amylose increased gradually in both varieties 13% and 10.6% in WH 896 and WH 912, respectively.
- Among all the biochemical parameters, the reduction under high temperature treatment was more in amylopectin in both the varieties.

GRAIN QUALITY CHARACTERS

- Starch, β-carotene, grain hardness, test weight, grain weight/5 spike and grain number/5 spike declined more in WH 912 than WH 896 under high temperature treatment.
- Total grain protein decreased in both the varieties under high temperature treatment. But the reduction was observed 17.1% more in WH 896 than WH 912 (3.1%).
- Grain shriveling score was observed more in WH 912 (78.0%) as compared to WH 896 (57.6%) under high temperature stress.
- Sedimentation value increased under high temperature treatment in WH 896 while decreased in WH 912.

- Among all the grain quality characteristics, the reduction was more in starch 31.4% and β-carotene content 39.7% in WH 912 under high temperature treatment.

Variety WH 896 was found more tolerant to heat stress than WH 912 when they were exposed to same degree of stress. Heat degree days were 852.5 and 1050.5 under control and high temperature conditions.

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ABSTRACT

Title of Thesis	**:**	**Starch metabolism and end use quality of durum wheat in response to high temperature stress**
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Key words : High temperature, durum wheat, starch metabolism, end use quality.

The present experimentation was carried out on durum wheat to study the physiological and biochemical basis of high temperature induced changes in sucrose to starch conversion. Effect of high temperature stress was determined on grain quality and starch metabolism in two durum wheat varieties WH 896 and WH 912. The investigation involved pot studies under screen house conditions during the year 2005-06.

In this study, high temperature was induced by shifting the pots to polyhouse after heading. Heat degree days (HDD) were used to quantity the magnitude of heat stress to which these two durum wheat

varieties were exposed during the second half of crop growth i.e. from days of heading (DOH) to days of physiological maturity (DOPM) and it is expressed as sum of daily mean temperature during particular period of crop growth. High temperature decreased functioning of various physiological parameters of the plants such as chlorophyll, chlorophyll fluorescence, chlorophyll stability index, membrane thermostability, osmotic potential and dry matter accumulation in grains. The detrimental effect of high temperature on various grain yield and quality characteristics such as grain shriveling score, grain hardness, grain weight/spike, grain number, test weight (1000-grains weight), β-carotene and total protein were also reflected. However, sedimentation value increased under high temperature treatment. Besides these, biochemical parameters such as total soluble sugars, reducing sugars, non-reducing sugars, protein, sucrose, starch and amylopectin were also reduced under high temperature stress except amylose. The enzymes and metabolites involved in starch biosynthesis also declined under high temperature treatment. Among enzymes, soluble starch synthase activity was reduced maximum as compared to other enzymes activities and among metabolites, ADP-glucose and UDP-glucose reduced maximum than other metabolites under high temperature stress.

Results of present experiment show that variety WH 896 is more tolerant to heat stress than WH 912 in manners of various physiological, biochemical and quality characteristics parameters when they were exposed to same degree of high temperature stress.

MAJOR ADVISOR **DEGREE HOLDER**

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