Association Between the Expression of Soluble Starch Synthase Family Members and Grain Weight in Wheat Under High Temperature Stress

NEERAJ JOSHI

DIVISION OF PLANT PHYSIOLOGY
INDIAN AGRICULTURAL RESEARCH INSTITUTE
NEW DELHI-110 012
2013
Association Between the Expression of Soluble Starch Synthase Family Members and Grain Weight in Wheat Under High Temperature Stress

BY

NEERAJ JOSHI

A Thesis
Submitted to the Faculty of the Post-Graduate School
Indian Agricultural Research Institute, New Delhi
In partial fulfilment of the requirements
For the award of the degree of

MASTER OF SCIENCE
In
PLANT PHYSIOLOGY

2013

Approved by:

Chairperson : Dr. Ajay Arora

Co-chairman : Dr. Rakesh Pandey

Members : Dr. Anil Dahuja
This is to certify that the thesis entitled “Association Between the Expression of Soluble Starch Synthase Family Members and Grain Weight in Wheat Under High Temperature Stress” submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfillment of MASTER OF SCIENCE in PLANT PHYSIOLOGY, embodies the results of bonafide research work carried out by Neeraj Joshi, Roll No. 20204 under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

Date: July 2, 2013
Place: New Delhi-12

(Ajay Arora)
Chairman
Advisory Committee
ACKNOWLEDGEMENT

It is by god’s grace and the will of the almighty that I have this opportunity to express my deep sense of gratitude to all those who directly or indirectly have helped me throughout the research work.

I wish to record my sincere innate respect, appreciation and gratitude to Dr. Ajay Arora, Principle Scientist, Division of Plant Physiology, IARI, New Delhi and the Chairperson of advisory committee, for providing me an opportunity to work under his able and highly exceptional guidance. I am thankful to him for his valuable guidance, wholehearted support and especially for the encouragement and confidence, he imparted to me throughout the period of my study and preparation of this manuscript.

My sincere thanks are also Dr. V. P. Singh, Professor, Division of Plant Physiology for providing me all the necessary facilities, guidance and encouragement throughout my research programme.

I owe a lot to my course teachers Dr. M. C. Ghildiyal, Dr. R. K. Sairam, Dr. P. S. Deshmukh, Dr. Vijay Paul, Dr. Poonam Natu, Dr. Pramod Kumar, Dr. Bhupinder Singh, Dr. Anjali Anand, Dr. Renu Pandey, Dr. Laeknixi, Dr. Akshay Shakare and other teachers and technical staff in the Division of Plant Physiology.

I also extend my sincere thanks to all the staff members of the Division of Plant Physiology especially Sh. S. N. Rai, Dr. Sitaram Kushwaha, Sh. Ashok, Sh. Umesh Thakur, Sh., Govind Banerjee, Sh. Devendra, Smt. Sunita Sharma and h. Chandeshwar for their kind cooperation and help during my research work.

I am deeply indebted to my lab mates and seniors Krishna Sir, Pranjali Ma’m, Shivani Ma’m, Deepika Ma’m, Suman Sir, Laxmi Ma’m, Shivram Sir, Krishn priya Mam, Shailes Sir, Vinith Sir, Surinder Sir, Sini Ma’m and Aradhna Ma’m for their cooperation, moral support, help, continued affection and unending encouragement and providing a congenial atmosphere in the laboratory during the course of this research work. I will always cherish the moments shared with them.
I express my deep sense of gratitude towards my family and more than a folks affiliate especially my parents, brother Kirti, Late Grandpa, Vipin and Meera, for their fond of love and best wishes for the advancement of my career, love, affection prayers and blessings received from them can-not be expressed in words.

And once and all I express my deep gratitude to the Great Almighty...
Dedicated to my parents
# CONTENTS

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1-5</td>
</tr>
<tr>
<td>2.</td>
<td>BACKGROUND</td>
<td>6-19</td>
</tr>
<tr>
<td>3.</td>
<td>MATERIALS AND METHODS</td>
<td>20-28</td>
</tr>
<tr>
<td>4.</td>
<td>RESEARCH PAPER</td>
<td>29-49</td>
</tr>
<tr>
<td>5.</td>
<td>DISCUSSION</td>
<td>50-54</td>
</tr>
<tr>
<td>6.</td>
<td>SUMMARY AND CONCLUSION</td>
<td>55-56</td>
</tr>
<tr>
<td>7.</td>
<td>ABSTRACT (ENGLISH)</td>
<td>57</td>
</tr>
<tr>
<td>8.</td>
<td>ABSTRACT (HINDI)</td>
<td>58</td>
</tr>
<tr>
<td>9.</td>
<td>BIBLIOGRAPHY</td>
<td>i-xvii</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>After Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>Temperature regimes during sowing, anthesis, 15DAA, 20 DAA and 25 DAA (From November 2012-March 2013).</td>
<td>21</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Effect of high temperature stress on leaf area per plant (cm²) in wheat genotypes at different growth stages.</td>
<td>39</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Effect of high temperature stress on total sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>40</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Effect of high temperature stress on reducing sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Effect of high temperature stress on non-reducing sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.5</td>
<td>Effect of high temperature stress on starch content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.6</td>
<td>Effect of high temperature stress on soluble starch synthase activity (nmol g⁻¹ dry weight min⁻¹) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.8</td>
<td>Effect of high temperature stress on grain growth rate (mg/day) in wheat genotypes.</td>
<td>43</td>
</tr>
<tr>
<td>4.4.9</td>
<td>Effect of high temperature stress on grain yield per plant (g) in wheat genotypes.</td>
<td>43</td>
</tr>
<tr>
<td>4.4.10</td>
<td>Effect of high temperature stress on test weight (g) in wheat genotypes.</td>
<td>44</td>
</tr>
<tr>
<td>4.4.11</td>
<td>Effect of high temperature stress on total biomass production per plant (g) in wheat genotypes.</td>
<td>44</td>
</tr>
<tr>
<td>4.4.12</td>
<td>Effect of high temperature stress on harvest index per plant (%) in wheat genotypes.</td>
<td>44</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES/PLATES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Title</th>
<th>After Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>Changes in maximum, minimum and mean temperature during grain development period of wheat genotypes.</td>
<td>21</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Effect of high temperature stress on leaf area per plant (cm²) in wheat genotypes at different growth stages.</td>
<td>39</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Effect of high temperature stress on total sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>40</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Effect of high temperature stress on reducing sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Effect of high temperature stress on non-reducing sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.5</td>
<td>Effect of high temperature stress on starch content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.6</td>
<td>Effect of high temperature stress on soluble starch synthase activity (nmol g⁻¹ dry weight min⁻¹) in grains of wheat genotypes at different growth stages.</td>
<td>41</td>
</tr>
<tr>
<td>4.4.7.1</td>
<td>RT-PCR expression analysis of <em>soluble starch synthase I</em> (SSSI) gene under high temperature stress and control conditions in wheat genotypes</td>
<td>42</td>
</tr>
<tr>
<td>4.4.7.2</td>
<td>RT-PCR expression analysis of <em>soluble starch synthase II</em> (SSSII) gene under high temperature stress and control conditions in wheat genotypes</td>
<td>42</td>
</tr>
<tr>
<td>4.4.7.3</td>
<td>RT-PCR expression analysis of <em>soluble starch synthase III</em> (SSSIII) gene under high temperature stress and control conditions in wheat genotypes</td>
<td>42</td>
</tr>
<tr>
<td>4.4.8</td>
<td>Effect of high temperature stress on grain growth rate (mg/day) in wheat genotypes.</td>
<td>43</td>
</tr>
<tr>
<td>4.4.9</td>
<td>Effect of high temperature stress on grain yield per plant (g) in wheat genotypes.</td>
<td>43</td>
</tr>
<tr>
<td>4.4.10</td>
<td>Effect of high temperature stress on test weight (g) in wheat genotypes.</td>
<td>44</td>
</tr>
<tr>
<td>4.4.11.</td>
<td>Effect of high temperature stress on total biomass production per plant (g) in wheat genotypes.</td>
<td>44</td>
</tr>
<tr>
<td>4.4.12</td>
<td>Effect of high temperature stress on harvest index per plant (%) in wheat genotypes.</td>
<td>44</td>
</tr>
</tbody>
</table>
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>adenosine 5’-triphosphate</td>
</tr>
<tr>
<td>AGPase</td>
<td>ADP-glucose pyrophosphorylase</td>
</tr>
<tr>
<td>BLAST</td>
<td>basic local alignment search tool</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>DAA</td>
<td>days after anthesis</td>
</tr>
<tr>
<td>DAS</td>
<td>days after sowing</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxyribonucleotide triphosphate</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetra-acetic acid</td>
</tr>
<tr>
<td>GBSS</td>
<td>granule bound starch synthase</td>
</tr>
<tr>
<td>GGD</td>
<td>grain growth duration</td>
</tr>
<tr>
<td>GGR</td>
<td>grain growth rate</td>
</tr>
<tr>
<td>HI</td>
<td>harvest index</td>
</tr>
<tr>
<td>HSP</td>
<td>heat shock proteins</td>
</tr>
<tr>
<td>kb</td>
<td>kilobase-pairs</td>
</tr>
<tr>
<td>kD</td>
<td>kilo dalton</td>
</tr>
<tr>
<td>LSD</td>
<td>least significant difference</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>ml</td>
<td>milli litre</td>
</tr>
<tr>
<td>mM</td>
<td>milli molar</td>
</tr>
<tr>
<td>m mol</td>
<td>milli mol</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>nmol</td>
<td>nano mol</td>
</tr>
<tr>
<td>NCBI</td>
<td>national centre for biotechnology information</td>
</tr>
<tr>
<td>°C</td>
<td>degree centigrade</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>RCA</td>
<td>rubisco activase</td>
</tr>
</tbody>
</table>
RNA  ribbonucleic acid
RnaseA  ribonuclease A
rpm  revolution per minute
RT-PCR  reverse transcription-polymerase chain reaction
SBE  starch branching enzyme
SEM  scanning electron microscopy
SOD  superoxide dismutase
SS  starch synthase
SSS  soluble starch synthase
SuSase  sucrose synthase
T6PS  trehalose-6-phosphate synthase
TBE  tris borate EDTA
TE  tris EDTA
Tm  melting temperature
Tris  2-amino-2-hydroxymethyl propane –1, 3-diol
UDP  uredine diphosphate
V/cm  volt/centimetre
WSC  water soluble carbohydrate
μg  micro gram
μl  micro litre
μM  micro molar
μmol  micro mol
1. INTRODUCTION

Climate change and abiotic stress affect agriculture and crop production adversely. Climate model projections suggest that higher seasonal temperatures will become commonplace in many parts of the world by the end of the century (Battisti and Naylor, 2009). Of the various climatic factors affecting agriculture, temperature is one of the most important, as higher temperatures adversely affect plant growth and yield (Kurek et al., 2007). Global mean surface temperature increased by around 0.5°C in the twentieth century and is projected to rise in a range from 1.8 to 4.5°C by 2100. As reported by De Costa (2011), the average global temperature is expected to rise by 1-6°C in the 21st century. There is evidence that minimum air temperatures across the globe have been “above normal” during several recent winter crop cycles (Ortiz et al., 2008). Winter crops are especially vulnerable to high temperature during reproductive phases. Researchers are engaged in understanding its impact on growth and yield of crops, and also identifying suitable management practices to sustain the crops’ productivity under the climate change scenarios (Aggarwal and Kalra, 1994).

Effect of global warming on six major food crops from the year 1982-2002, revealed combined yield loss of around 40 million tonnes for wheat, corn and barley per year, where wheat alone accounts for almost half of the yield loss (19 mt year⁻¹) (Lobell and Field, 2007). At the global level, the demand for wheat is growing at approximately 2% per year (Rosegrant and Cline, 2003). However, genetic gains in yield potential of wheat stand at less than 1% (Sayre et al., 1997). Consequently, yield plateau has been observed in the last decade and has been attributed to many factors among which high occurrence of terminal heat stress appears to be the most prominent (Nagarajan, 2005). A significant proportion of wheat grown in South Asia experiences heat stress out of which a major portion is present in India (Joshi et al., 2007a).

The recent succession of years with above average temperatures has increased concerns about the impact of heat stress on crop yields including those of wheat (Triticum aestivum L.). Wheat cultivation in India started 5000 years ago (Feldman, 2001). It is a major crop of India and used as a staple food in the country and as per 2nd Advance Estimates, the production of wheat during the year 2012-13 is estimated
at 92.30 million tonnes (Department of Agriculture and cooperation, Ministry of India). India is among the top ten wheat producing countries of the world. In terms of area under cultivation it is positioned at number one whereas in terms of production it is ranked second next to China (FAOSTATS, 2010).

Wheat is grown in India under sub-tropical environment during mild winter which warms up towards grain filling stages of the crop. Since nearly 90% area under wheat in India has access to irrigation, yield is limited by supra-optimal temperatures prevailing during various crop growth stages. Almost all stages of wheat growth and development are adversely affected by heat stress. It has long been known that average wheat yield reduces by 4% for every one degree rise in ambient temperature during grain filling (McDonald et al., 1983). Even though optimum temperature for grain growth in wheat is 15ºC (Chowdhury and Wardlaw, 1978), moderately high temperatures (25-32ºC) for longer duration and very high temperatures (33-40ºC) for a shorter period are commonly experienced in Mediterranean and subtropical environments, particularly during grain filling (Stone and Nicolas, 1994). High temperature is a main abiotic stress that harmfully influences both vegetative and reproductive growth.

One of the most obvious impacts of temperature increase on wheat is earlier occurrence of phenological stages (Porter and Gawith, 1999). Heat stress induce decrease in the duration of developmental phases leading to fewer organs, smaller organs, reduced light perception over the shortened life cycle and perturbation of the processes related to carbon assimilation (transpiration, photosynthesis and respiration) are significantly contributing to losses of yield. Terminal heat stress is a major abiotic stress affecting yield in wheat, and genetic diversity for heat tolerance has been reported (Al-Khatib and Paulsen 1990; Joshi et al., 2007a). The photosynthetic process is affected under heat stress conditions, defined by Fischer and Byerlee (1991) to be mean daily temperatures of 17.5ºC in the coolest month, especially during grain filling when demand for assimilates is the greatest. Earlier and recent evidence has indicated that short periods (3-5 days) of high temperature (>35ºC) stress during the grain filling stage of wheat occurs quite often (Semenov, 2009) which may result in significant reductions of both grain yield and quality (Stone and Nicolas, 1995).
Carbon metabolism in plants is one of the key physiological processes determining crop growth, yield and quality; and is very sensitive to abiotic stresses. In the field, unfavorable environmental conditions can occur at any time in the life cycle of the wheat plant, can vary in intensity and duration, and can affect diverse processes during either vegetative or reproductive phases of the plant. The plant has devised numerous mechanisms to cope with environmental stress with the ultimate goal of producing a viable seed. Tolerance to heat stress is a complex phenomenon and controlled by multiple traits/genes imparting a number of physiological and biochemical changes such as alteration in membrane structures and function, tissue water content, composition of protein, lipids, primary and secondary metabolites and no single trait fully explains why some wheat varieties are able to give better yield even when they experience heat stress. Tolerance mechanisms involve changes at the molecular, cellular and physiological levels that vary with genotype and are further influenced by the nutritional status of the plant. Ultimately, changes are manifested in the grain where they influence the accumulation of starch and protein. Starch is a major component of the grain and is the most important factor for yield while both the amount and composition of protein are critical for quality (Altenbach, 2012). Accumulation of starch is more sensitive than is the deposition of protein (Bhullar and Jenner, 1985), and it is the conversion of sucrose to starch within the developing endosperm that is decreased by elevated temperature (Bhullar and Jenner, 1986).

Grain filling is the final stage of growth in cereals where fertilized ovaries develop into caryopses. Its duration and rate determine the final grain weight, a key component of the total yield. In today’s crop production systems with their high yield outputs, improvement in grain filling has become more challenging than ever (Zahedi and Jenner, 2003). The grain-filling rate of wheat, like other cereals, depends on two main sources of carbon: current assimilates from photosynthesis and stored water soluble carbohydrates (WSCs) transported to the grain from leaves, stem and ear reserves (Gent, 1994; Yang and Zhang, 2006). The relative importance of the two carbon sources in contributing to the grain filling varies among genotypes and is often dependent on environmental conditions (Blum, 1998). Under heat stress, crop photosynthesis declines and is unable to support both respiration and grain growth (Gent, 1994) and the relative importance of stored WSC to sustain grain growth becomes greater. Sustained periods of high temperature (30-38°C) from
anthesis to maturity have been found to reduce grain yields mainly by reducing grain growth, but the magnitude of the effect can vary from 20 to 50% (Wardlaw et al., 1989a; Tewolde et al., 2006). Starch is a major component of the wheat grain, making up 60-70% of its dry weight. An earlier study has indicated that decline in starch content in cereal grains is responsible for the decrease in grain yield under heat stress (Labuschagne et al., 2009). High temperatures during wheat grain filling stage reduced starch content and altered the size distribution of starch granules in the mature grains, reduce the final weight of the wheat grain, and diminish yield (Hurkman et al., 2003).

On the sink side, grain filling is a process of active metabolism of carbohydrate and starch accumulation in kernels. It is generally accepted that four enzymes may play a key role in this process: Sucrose Synthase (SuSase), ADP glucose pyrophosphorylase (AGPase), starch synthase (SS), and starch branching enzyme (SBE) (Hawker and Jenner, 1993; Ahmadi and Baker, 2001; Hurkman et al., 2003). Soluble starch synthase (SSS) enzyme activity is reported to be positively correlated with the rate of starch synthesis in wheat grains (Keeling et al., 1993). In wheat, immediately after the heat shock, the activities of both the soluble and bound starch synthase were significantly decreased (Rijven, 1986). Reduction in grain growth rate of water-stressed wheat plants resulted from reduced starch synthase activity, whereas growth cessation resulted mainly from the inactivation of AGPase (Ahmadi and Baker, 2001). However, AGPase was not found to be sensitive to moderate heat so as to be responsible for decreased starch accumulation and grain growth under late sowing. Sumesh et al. (2008) studied starch synthase activity and heat shock protein in relation to thermal tolerance of developing wheat grains. The grain yield components were determined and found that there was higher catalytic efficiency ($V_{\text{max}}/K_m$) of soluble starch synthase and higher expression of HSP100 at elevated temperature.

Biochemical studies of starch biosynthesis in the developing wheat grain indicate that high temperature decreases metabolite levels and enzyme activities associated with this pathway. The analysis reveals that the first step in this pathway, catalysed by sucrose synthase, is altered at high temperature and other observations are indicative of effects also at the level of starch synthase (Jenner, 1994). Temperatures of more than 25°C adversely affect the activity of SSS, an
amyloplastic enzyme, in endosperm of wheat. Enzyme rate was found to have a temperature optimum between 20°C to 25°C (Keeling et al., 1993).

Very little information is available on changes in the activities of key enzymes in the sucrose-to-starch catalytic pathway in high temperature stressed wheat grains during the grain-filling period. Keeping these facts in mind the present investigation was planned with the following objective:

1. Studies on the expression and activity of soluble starch synthase in grains of wheat genotypes with contrasting thermotolerance under high temperature stress.
2. BACKGROUND

Introduction

Gaseous emissions due to human activities are substantially adding to atmospheric concentrations of greenhouse gases. In the atmosphere these gases trap heat radiated from the earth and thus increase global mean temperature. This rise in temperature may lead to altered geographical distribution and growing season of agricultural crops by altering the threshold temperature for the start of the season and crop maturity (Porter, 2005). High temperatures negatively affect plant growth and survival and hence crop yield (Boyer, 1982). According to a study (Lobell and Asner, 2003) each degree centigrade increase in average growing season temperature may reduce crop yields up to 17%. From 1950 to 1993, the increase in global daily minimum temperatures was more than double the increase in daily maximum temperatures (Easterling et al., 1997). To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how tolerance to heat can be improved (Halford, 2009). Under field conditions, high temperature stress is frequently associated with reduced water availability (Simoes-Araujo et al., 2003). Thus, high temperature is one of the most important climatic factors that adversely affect plant growth and yield (Kurek et al., 2007). Plants detect changes in ambient temperature through perturbations in metabolism, membrane fluidity, protein conformation and assembly of the cytoskeleton (Ruelland and Zachowski, 2010). Such reactions activate adaptive processes like expression of heat shock proteins, until new cellular equilibriums are reached. However, temperatures above the optimum for growth can be deleterious, causing injury or irreversible damage, which is generally called ‘heat stress’. Heat stress is a function of the magnitude and rate of temperature increase, as well as the duration of exposure to the raised temperature (Wahid et al., 2007).

Wheat (Triticum aestivum L.) is the most important staple food of about two billion people. Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). In India, wheat productivity is the highest in fertile Indo-Gangetic plains of North India under irrigated
conditions, reaching a high of about 6-7 t ha\(^{-1}\), while in Central India maximum yield ranged between 3-4 t ha\(^{-1}\) only. These differences in yield are mainly related to higher temperatures and longer day lengths prevailing in Central India during crop growth period as compared to North India (Ruwali and Prasad, 1991). Trends in increasing growing season temperatures have already been reported for the major wheat-producing regions (Gaffen and Ross, 1998; Alexander \textit{et al.}, 2006). Wheat experiences heat stress to varying degrees at different phenological stages, but heat stress during the reproductive phase is more harmful than during the vegetative phase due to the direct effect on grain number and dry weight (Wollenweber \textit{et al.}, 2003). End-of-season or ‘terminal’ heat stress is also likely to increase for wheat in the near future (Mitra and Bhatia, 2008; Semenov, 2009). Hence, the main focus is on responses to elevated temperatures during reproductive and grain-filling stages and processes that affect grain yield.

Exposure to temperatures above optimum can significantly reduce grain yield (McDonald \textit{et al.}, 1983; Macas \textit{et al.}, 2000; Mullarkey and Jones, 2000; Tewolde \textit{et al.}, 2006) and hastens physiological maturity, i.e., shortening the grain filling period in bread and durum wheat (Dias and Lidon, 2009). In wheat, the threshold temperature for heat stress is found to be 26°C, which occurs at post-anthesis. Above this threshold a detectable reduction in growth and yield begins (Stone and Nicolas, 1994). Temperature rises beyond a threshold (5-10°C above ambient) may cause irreversible damages to plant function and development or alteration of metabolism, resulting in reduction in growth and yield (Porter, 2005).

There have been significant differences within bread and durum wheat species concerning the capability to cope with high temperatures at the stage of grain filling (Dias \textit{et al.}, 2010). Increased daily minimum temperature appears to have greater impact on wheat production as grain yield is more strongly negatively correlated with increasing minimum temperatures than maximum temperatures (Lobell \textit{et al.}, 2005). Night temperatures >20°C can reduce spikelet fertility with a concomitant reduction in grain number and size. Increased night temperatures also linearly decrease the duration of grain filling. Prasad \textit{et al.} (2008a) found that night temperatures of 20 and 23°C
reduced the grain-filling period by 3 and 7 days, respectively. Significant variations were seen among cultivars in the reduction in grain weight per ear, kernel number, and single kernel weight under heat stress (Fokar et al., 1998). Late sowing in wheat reduced the grain filling in various genotypes due to the exposure to high temperature during grain filling stage (Pandey and Srivastava, 2009). Although high temperatures accelerate growth (Fischer, 1980; Kase and Catsky, 1984), they also reduce the phenology, which is not compensated for by the increased growth rate (Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003). However, temperatures >30°C, during floret formation, may cause complete sterility (Saini and Aspinal, 1982). Heat stress during the reproductive phase can cause pollen sterility, tissue dehydration, lower CO₂ assimilation and increased photorespiration. Therefore, when temperatures are elevated between anthesis to grain maturity, grain yield is reduced because of the reduced time to capture resources.

**Photosynthesis**

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme that regulates carboxylation during photosynthesis (Ogren, 1984). The inhibition of photosynthesis due to high temperature is often attributed to increases in the rate of photorespiration. This is because the solubility of CO₂ and O₂, and the kinetics of Rubisco are affected under high temperatures (Ogren, 1984; Long et al., 2004). Upon exposure to high temperature, Rubisco is deactivated because of faster rate of end product formation or slower reactivation by Rubisco activase (RCA), resultantly RCA looses its ability in keeping the Rubisco active and efficient (Salvucci and Crafts-Brandner, 2004a). Demirevska-Kepova et al., (2005) observed changes in the abundance of Rubisco large and small subunits and RCA in wheat leaves upon exposure to heat stress (40°C) in darkness or in light. Heat stress for 24 h in darkness irreversibly decreased the Rubisco subunits and RCA. Demirevska-Kepova et al., (2005) reported Heat stress for 24 h in darkness irreversibly decreased the Rubisco subunits and RCA. At high temperatures, the solubility of oxygen decreases to a lesser extent than CO₂, resulting in increased photorespiration and lower photosynthesis (Lea and Leegood, 1999).
In many plant species, the ability to sustain leaf gas exchange under heat stress has a direct relationship with heat tolerance. During the vegetative stage, high day temperature can cause damage to compensated leaf photosynthesis, reducing CO₂ assimilation rates (Hall, 1992). According to Sage and Kubien, (2007) limitations in electron transport and RCA capacity is expected to be more common in the warmer, high CO₂ conditions by the end of the century. Cell membrane thermo-stability can be related to the capacity for sustained grain filling under heat stress (Fokar et al., 1998). The increased solute leakage, as an indication of decreased cell membrane thermo-stability (CMT) has long been used as an indirect measure of heat-stress tolerance in diverse plant species including wheat (Blum et al., 2001).

**Dry matter accumulation**

The rate of dry matter accumulation (i.e. import) is determined by sink strength which is a product of sink size and the metabolic activity of the sink organ during development (Ho, 1988). Under low to moderate heat stress, a reduction in source and sink activities may occur leading to severe reductions in growth, economic yield and harvest index. High temperature significantly affects assimilate partitioning via apoplastic and symplastic pathways (Taiz and Zeiger, 2006). However, considerable genotypic variation exists in crop plants for assimilate partitioning (Yang et al., 2002). To elucidate causal agents of reduced grain filling in wheat under high temperatures, Wardlaw (1974) examined three main components of the plant system including source (flag leaf blade), sink (ear), and transport pathway (peduncle). It was determined that photosynthesis had a broad temperature optimum from 20-30°C, however, it declined rapidly at temperatures above 30°C. The rate of \(^{14}\text{C}\) assimilate movement out of the flag leaf (phloem loading), was optimum around 30°C, however, the rate of movement through the stem was independent of temperature from 1-50°C. It was concluded that, at least in wheat, temperature effects on translocation result indirectly from temperature effects on source and sink activities.
**Flower and embryo development**

Temperatures above 30°C during floret formation cause complete sterility (Saini and Aspinall, 1982). The numbers of kernels per unit area decreases at a rate of 4% for each degree increase in mean temperature during the 30 d preceding anthesis (Fischer, 1985). Floral abnormalities induced by heat stress (i.e. stamen hypoplasia and pistil hyperplasia), leading to spikelet sterility, represent significant problems in rice production (Takeoka et al., 1991). Chebotaru (1965) noted an increase in the number of antipodal cells in the maize embryo sac as a result of high temperature. Saini and Aspinall (1982) observed that a level of heat stress that caused male sterility in wheat had no damaging influence on the functions of female sexual generation, suggesting that the female gametophyte had greater heat stress tolerance. When the Australian wheat variety ‘Gabo’ was subjected to high temperature (30°C) during meiosis, a third of the ovaries were found to exhibit abnormal development (Saini et al., 1983).

**Pollen formation**

Pollen formation is one of the most heat-sensitive developmental stages in cereals (Saini and Aspinall 1982; Stone 2001). Pollen grain mitosis 1 and 2 are highly sensitive to elevated temperature both in wheat (Saini et al., 1984) and barley (Sakata et al., 2000). In wheat, two types of abnormal pollen development can be caused by high temperature stress. The first is apparently caused by tapetal degradation during meiosis, when the microspores are notable to complete the first mitosis. They may have an exine but no cytoplasm, and may remain immature. In the second case, all the microspores complete the first mitotic division, but only a few of them are able to divide further to develop into normal tri-cellular pollen grains. The rest of the microspores remain immature and do not accumulate starch, so the anthers contain a mixture of fertile and sterile pollen grains (Saini et al., 1984). Failure of mature and germinating maize pollen to respond to heat stress has been observed for many Heat Shock proteins (HSPs), such as HSP60, HSP70 and HSP90 (Hopf et al., 1992; Magnard et al., 1996) and correlates with its pronounced loss of viability when exposed to elevated temperatures (Herrero and Johnson 1980; Mitchel and Petolino 1988; Dupuis and Dumas 1990). These
observations suggest the sensitivity of pollen to heat stress is associated with the inability of pollen to synthesize HSPs (Mascarenhas and Crone, 1996). In maize, reduction in seed set occurs at temperatures higher than 38°C mainly because of a reduction in pollen germination ability and pollen tube elongation (Dupuis and Dumas, 1990; Stone, 2001). Increased temperature over the mid-anthesis period decreased the grain number per ear at maturity in spring wheat indicating the heat sensitivity of fertilization and grain setting (Ferris et al., 1998). Thus, heat stress during anthesis increases floret abortion (Wardlaw and Wrigley, 1994).

**Grain filling**

Both grain number and weight are sensitive to elevated temperature (Ferris et al., 1998). Influence of temperature on each of these components of grain yield depends on the developmental phase at which the elevated temperature occurs. For instance, between spike initiation and anthesis, temperatures above 20°C may substantially reduce grain number per spike (Saini and Aspinall, 1982). Heat stress speeds up development of the spike (Porter and Gawith, 1999) reducing spikelet number and thus, the number of grains per spike (Saini and Aspinall, 1982). The most sensitive period is between the appearance of double ridges on the shoot apex and flag leaf. An inverse relationship between duration of heat stress and grain number per spike has been observed during this time (Rawson and Bagga, 1979). The reason for this sensitivity is because spikelets begin to form in the spike from ridges of tissue between ridges of undifferentiated leaf primordia, called the double ridge stage. The reduction in the duration of emergence to double ridge and double ridge to anthesis reduces spikelet number per spike and grain number per spikelet (McMaster, 1997). Grain number per spike decreased by 4% for every 1°C (from 15-22°C) increase in the 30 days preceding anthesis (Fischer, 1985). The availability of carbohydrates for floret development is one factor determining grain number (Abbate et al., 1995; Demotes-Mainard and Jeuffroy, 2004) because inadequate availability of assimilates may cause floret death (Kirby, 1988). Wheat plants exposed to 30°C for 3 consecutive days, when pollen mother cells were dividing, substantially reduced grain set and therefore grain yield. Reduction in grain set was also observed.
when plants were exposed for 1 day to 30°C, or for 3 days to day/night temperatures of 30/20°C. Reductions in grain yield due to reduced grain set are not compensated for by increases in grain weight (Saini and Aspinall, 1982). Reduced grain weight (nearly 1.5 mg per day) can occur for every 1°C above 15-20°C (Streck, 2005). Variability in terms of high temperature effects on wheat grain number and size appears to be related to genotypic differences in heat tolerance (Viswanathan and Khanna-Chopra, 2001; Tahir and Nakata, 2005). Increase in temperature (from 30-38°C), during reproductive phase, reduced the main stem grain weight by 20-44% (Tahir and Nakata, 2005). Elevated temperatures can also cause grain shrinkage through ultrastructural changes in the aleurone layer and endosperm cells (Dias et al., 2008) when day/night temperatures increased from 25/14°C to 31/20°C.

Heat stress accelerates the rate of grain filling whereas grain filling duration is shortened (Dias and Lidon, 2009). For instance, 5°C increases in temperature above 20°C increased the rate of grain filling and reduced the grain filling duration by 12 days in wheat (Yin et al., 2009). Under these conditions, the supply of photoassimilates may be limited (Calderini et al., 2006). It is estimated that for every 1°C above the optimal growing temperature of 15-20°C, the duration of grain-filling is reduced by 2.8 days (Streck, 2005). The rate of grain growth increases as temperature increases, but this apparently depends on whether the number of grains per spike is reduced. In spikes where the number of grains is less affected by elevated temperature, spikelets reduce the rate of grain growth. Therefore, we would expect that an increase in the grain filling rate could compensate for the shorter grain-filling period; however, this did not occur at temperatures above 30°C (Sofield et al., 1977). Other studies have also reported that the duration of grain filling under heat stress was not compensated by greater grain filling rates (Wardlaw et al., 1980; Stone and Nicolas, 1995). Both duration and rate of grain growth were reduced by heat stress in genotypes differing in grain weight stability (Viswanathan and Khanna-Chopra, 2001). Heat stress during grain filling phase affects the grain protein contents (Wardlaw et al., 2002; Gooding et al., 2003) through reductions in starch deposition, which influences protein concentration by allowing more nitrogen per unit of starch (Stone and Nicolas, 1998). Although the daily flow of carbon
and nitrogen into grain increases with increasing temperature, carbon flow decreases per degree day (Wardlaw et al., 1980; Daniel and Triboi, 2000). As a result, grain size is more affected by temperature than quantity of grain nitrogen (Uhlen et al., 1998; Daniel and Triboi, 2000). Grain protein content is inversely related to grain size (Guttieri et al., 2000; Erekul and Kohn, 2006). Total grain protein content of the crop decreases under heat stress because heat stress decreases grain yield (Stone and Nicolas, 1998; Castro et al., 2007). Heat stress also decreases the duration, but not rate, of protein deposition in the grain. The protein content in wheat occurs when heat stress is imposed early in grain filling (Castro et al., 2007).

**Starch biosynthesis**

Biochemical conversion of sucrose to starch is one of the most important components of sink strength and can be determined by the catalytic activities of one or more of the enzymes involved in this pathway. Starch in the endosperm of wheat is the major form of carbon reserves and comprises 65-75% of the final dry weight of the grain (Housley et al., 1981; Dale and Housley, 1986; Hurkman et al., 2003) and reduced starch deposition is the main reason of reductions in grain weight (Bhullar and Jenner, 1985). As the enzymes involved in starch biosynthesis, in developing kernels of wheat, are sensitive to high temperature, the process is sensitive to heat stress (Denyer et al., 1994; Jenner, 1994). As temperatures rise above 18-22°C, the duration of starch biosynthesis and deposition to grain is reduced. Reductions in starch content account for most of the reduction in grain dry matter at temperatures above 18-22°C (Spiertz et al., 2006).

Grain filling is mainly a process of starch biosynthesis and accumulation. It is generally accepted that four enzymes may play a key role in this process: Sucrose Synthase (SuSase), ADP Glucose Pyrophosphorylase (AGPase), Starch Synthase (SS), and Starch Branching Enzyme (SBE); (Hawker and Jenner, 1993; Ahmadi and Baker, 2001; Hurkman et al., 2003). In the process of starch synthesis, SuSase catalyses the cleavage of Sucrose, the main transported form of assimilates in wheat plants (Fisher and Gifford, 1986), to form UDP-Glucose and Fructose, which is thought to be the first
step in the Sucrose-to-starch conversion. AGPase produces ADP-Glucose, the primer of the starch chain (Smith and Denyer, 1992), and is regarded as the rate-limiting enzyme in starch biosynthesis (Preiss, 1988).

SS, composed of both Soluble and Granule-Bound (GBSS) isoforms, elongates the amylose and amylopectin chains (Dejardin et al., 1997). Soluble Starch Synthase
SSS) activity is reported to be positively correlated with the rate of starch synthesis in wheat grains (Keeling et al., 1993). SBE forms branches on the polymers. It cleaves \(\alpha-1,4\) bonds on both amylose and amylopectin molecules and reattaches the released glucan segments to the same or another glucan chain through the formation of \(\alpha-1,6\) linkages (Hurkman et al., 2003). Its activity is closely associated with the increase in starch content during the development of rice endosperm (Nakamura et al., 1989). It has been assumed that GBSS functions specifically to elongate amylose, while SSS contributes to amylopectin synthesis. SSS regulates the synthesis of starch and is sensitive to heat stress (Rijven, 1986; Keeling et al., 1993). Heat stress decreases the activity of SSS in wheat, reducing grain growth and starch accumulation (Prakash et al., 2003, 2004). Even short periods of episodic temperature over 30ºC slows starch accumulation principally due to heat induced denaturation of SSS (Jenner, 1994).

The relative importance of any one of these enzymes in this respect may be dependent on plant type. In wheat endosperm, however, it is the catalytic activity of SSS, and not AGPase, which is thought to be the important factor controlling the rate of synthesis of starch (Jenner and Hawker, 1993; Keeling et al., 1993). SSS activity is positively related to dry matter accumulation in tomato fruits (Demnitz-King et al., 1997). SSS activity was reported to be higher in wheat kernels achieving greater maximum dry weight (Dale and Housley, 1986). The duration of SSS activity is believed to be important in determining the duration of grain filling (Chevalier and Lingle, 1983). A decline in SSS activity was reported under high temperature in heated grains of wheat and decline was highly correlated with the decline in rate of starch synthesis (Hawker and Jenner, 1993; Jenner et al., 1993; Keeling et al., 1993). In maize kernels cultured in vitro, among the several enzymes of starch synthesis assayed, only AGPase showed a marked response to heat stress (Duke and Doehlert, 1996). MacLeod and Duffus (1988) reported that reduction in grain weight in response to heat stress was due to reduced sucrose synthase activities. In barley starch accumulation is reduced when endosperms develop at elevated temperatures. When developing ears are exposed to elevated temperatures, there is an irreversible reduction in the capacity of the endosperm to convert sucrose to starch, caused by a decrease in the activity of at least one of the
enzymes involved in the conversion pathway (MacLeod and Duffus, 1998). Ahmadi and Baker (2001) reported that under more severe stress conditions (PEG treated detached ears) the cessation of dry matter accumulation corresponded to a nearly complete inhibition of AGPase activity while SSS showed 55% of the activity of the control grains and GBSS was not affected. He has also reported that among these regulatory enzymes in the biochemical pathway of sucrose to starch, SSS, GBSS and AGPase are more likely to be affected by water stress, than the other two enzymes. Among these enzymes, SSS with the lowest catalytic activity and highest susceptibility appeared to be a major site of regulation of starch synthesis in the developing wheat grain, and the first enzyme to be affected by stress conditions (Ahmadi and Baker, 2001). Diminished rates of starch production in wheat endosperm are also reported to be due to heat inactivation of starch synthase, a key enzyme in the starch biosynthetic pathway (Labuschagne et al., 2009).

Four isoforms of starch synthase, SSSI, SSSII, SSSIII, and GBSS I have been shown to be expressed in the wheat endosperm during grain filling (Denyer et al., 1995). Activities of SSS and GBSS peaked at 20 and 24 DAA, respectively. In wheat SSS I was generally expressed over the grain filling stage; the SSSII and SSSIII were expressed over the early and mid-grain filling stage, and the GBSS I was expressed during the mid to late grain filling stage (Zhang et al., 2010). SSSI is primarily responsible for the synthesis of the shortest chains, and further extension of longer chains is achieved by SSSII and SSSIII in amylopectin (Craig et al., 1998; Gao et al., 1998; Umemoto et al., 2002). GBSS I is not only responsible for the synthesis of amylose, but also lays roles for biosynthesis of extra-long unit chains of amylopectin (Hanashiro et al., 2008). However, each isoform contributes differently to the activity of starch synthase. For instance, SSSII and SSSIII account for more than 60% and 80% of the soluble starch synthase activity in pea embryo and potato tuber, respectively (Craig et al., 1998). On the other hand, SSSI is the major contributor to the total soluble starch synthase activity in wheat endosperm and accounts for almost two-thirds of activity (Peng et al., 2001).

In spite of a high temperature exposure during grain development, the maize grains had a remarkably higher (3-4 times high) SSS activity as compared to wheat.
GBSS activity was considerably lower than SSS activity, indicating SSS is the major enzyme for starch synthesis. High temperature exposure of excised developing grains showed no significant decrease in SSS activity in maize, whereas, *T. aestivum* (var. Lok Bold) showed a significant decrease. This shows that an efficient and relatively thermostable SSS is present in maize (var. HQPM7) as compared to wheat, which could possibly be utilized in improving thermotolerance for grain growth in wheat (Pandey *et al.*, 2012)

Under mean daily temperature of 32°C (high temperature) and 22°C (normal temperature) controlled in growth chambers, the expression responses of 8 SSS isoform genes involving starch synthesis metabolism in rice. The expression patterns of SSS genes under high temperature stress were isoform-dependent, in which some isoform genes, such as *SSSIlb*, *SSSIlc*, *SSSIib*, and *SSSIVa*, were up-regulated with relatively high expression levels, and other genes, such as *SSSIia* and *SSSIia*, showed down-regulated patterns with relatively low expression levels. SSS I and SSS IIIa were highly expressed in rice endosperms during the whole filling period in both temperature treatments, implying that they are the major isoform genes of SSS. The expressions of *SSSIlb*, *SSSIia*, and *SSSIVa* genes were much more sensitive to high temperature stress than those of *SSSI*, *SSSIic*, *SSSIib*, and *SSSIVb* (Wei *et al.*, 2009)

Prakash *et al.* (2009) studied in Wheat cvs. HD 2285 and C 306 (relatively tolerant) and HD 2329 and HD 2428 (susceptible type) were exposed during grain development period to 5-7°C higher temperature in a normal glasshouse and 8°C higher than control in temperature control glasshouse of phytotron facility. There was more severe depression in grain growth under elevated temperature in phytotron experiment and was associated with greater decrease in the activity of soluble starch synthase in the grains. Furthermore, relatively tolerant cultivars showed less decrease compared to susceptible cultivars in grain growth and also showed less decrease in SSS activity. Such parallelism in the effect of elevated temperature on grain growth and SSS activity and relatively tolerant cultivars showing less depression, further suggests that soluble starch synthase is the key component imparting sensitivity to high temperature for grain growth
in wheat. The study suggested that thermotolerance for grain growth in wheat could possibly be improved through incorporation of thermostable form of this enzyme.

**Tolerance mechanism under high temperature stress**

Plants exposed to high temperature are also induced to synthesize HSPs. HSPs are known to function as molecular chaperones that aid in refolding proteins denatured by heat and prevent them from aggregating (Boston *et al.* 1996, Iba 2002, Vierling, 1991). Wahid and Close (2007) reported expression of dehydrins under heat stress in sugarcane. Accumulation of high molecular mass HSPs has been directly implicated in induction of thermotolerance in plants (Boston *et al.* 1996). Katiyar-Agarwal *et al.* (2003) introduced *Arabidosis thaliana* HSP 101 (*Athsp* 101) cDNA into Pusa Basmati cultivar of rice. The transgenic rice lines showed significantly better growth performance in the recovery phase following the heat stress. The HS100 kDa protein has been reported to have a definite role in thermotolerance (Lee *et al.* 1994, Schirmer *et al.* 1994).

Wheat cvs. HD 2285 (relatively tolerant) and WH 542 (susceptible) were exposed to ambient and elevated temperature (3-4°C higher) in open top chambers during post anthesis period. In order to elucidate the basis of differential tolerance of these cultivars, the excised developing grains (20 DAA) of ambient grown plants were incubated at 15, 25, 35 and 45°C for 2 h and then analysed for the activities of SSS, GBSS, kinetic parameters of SSS and content of heat shock protein (HSP 100). The elevated temperature during grain development significantly decreased grain growth in WH 542 whereas no such decrease was observed in HD 2285. High temperature tolerance of HD 2285 was found to be associated with higher catalytic efficiency ($V_{\text{max}}/K_m$) of SSS at elevated temperature and higher content of HSP 100 (Sumesh *et al.*, 2008).

Protein profiling and isoenzymes analysis showed the expression of several heat-stable proteins and prominent isoenzymes of SOD in C-306 (thermotolerant), compared to PBW 343 (thermosusceptible), cultivars of wheat. Scanning electron microscopy (SEM) of starch granules showed globular, well-shaped and more numbers of
endospermic cells in C-306, compared to defragmented, irregular shaped and shrunken granules in case of PBW 343 under heat stress treatment (42°C for 2 h). Diurnal change in SSS activity showed an increase in the activity during afternoon (35°C), compared to morning (29°C) and evening (32°C) in both the cultivars. Under heat stress (42°C for 2 h), a drastic decrease in the SSS activity was observed, due to the thermal denaturation of the enzyme. Thermotolerance capacity analyzed using cell membrane stability (CMS) showed significantly higher CMS in case of C-306, compared to PBW343 at different stages of growth. Findings suggest that abundance of HSPs and SODI during milky-dough stage plays a very important role in starch granule biosynthesis (Kumar et al., 2013).
3. MATERIALS AND METHODS

The present study was conducted in the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi (latitude 28°N and longitude 77°E, and about 250m above mean sea level). The plants were grown in the pot culture, Division of Plant Physiology, in winter rabi season during 2012-13, with recommended cultural practices. The experiments were also conducted in Polyhouse, IARI, New Delhi, by shifting pots to polyhouse after anthesis, for the imposition of high stress treatment high temperature stress treatment.

3.1. Plant Material

Five wheat genotypes were used for the experiment as suggested by the breeders on the basis of thermotolerance. The genotypes were obtained from Division of Genetics, IARI, New Delhi.

1. PBW 550
2. Raj 3765
3. KAUZ
4. HD 2733
5. WH 730

Sowing was done in earthen pots (about 30 cm in diameter and 30 cm in depth) filled with clay loam soil and farmyard manure (FYM) in 3:1 ratio during *rabi* (winter) season. Nitrogen, phosphorus and potash fertilizers were applied at the rate of 60: 60: 60 kg per hectare, respectively in the form of urea, single super phosphate and muriate of potash at the time of sowing. Remaining 60 kg N ha⁻¹ was given 25 days after sowing.

3.2. Experimental treatment

The genotypes were raised in pot culture of Division of Plant Physiology, with date of sowing of 24th November, 2012. Ten seeds per pot were used at the time of sowing. On 17th December, 2012 thinning in each pot was done keeping four plants per pot. Half of the pots from each genotype were shifted to polyhouse around 13th February, 2013 (nearly a week prior to anthesis). In polyhouse the heat stress treatments
were given with average of 32.4°C/15.5°C (difference of 2.6°C/1.5°C compared to ambient day/night temperature) at the time of grain filling (Table 3.2 and Plate 3.2).

Schedule routine of irrigation was maintained for control as well as stress treated plants.

3.3. Sample collection

Plants were sampled and observations were recorded for growth, physiological and biochemical parameters. Tagging was done from the date of anthesis observed (Plate 3.2). Grain samples were collected from control and high temperature stressed plants as per the tagging done, at 15 days after anthesis (DAA), and further at 5 days interval from first sampling (20 and 25). At each stage samples were collected in triplicate from three pots. For gene expression studies grain samples were used for total RNA extraction, which was done by storing the samples at -80°C.

3.4. Observations recorded

3.4.1. Growth parameters

1. Leaf area

The leaves were separated from the stem and cleaned thoroughly. All the green leaves from each replication (three plants per replication) were taken. Leaf area of flag leaf, main shoot leaves and total plant leaf area was measured using a standard leaf area meter (Model LiCOR 3100) and was expressed as cm² plant⁻¹.

2. Grain growth duration (GGD)

3. Grain growth rate (GGR)

Grain growth was recorded by taking grains at 15 DAA and then at an interval of every 5 days up to 30 DAA. Grain growth curves were made using Microsoft Office Excel, 2007. The point on the curve where grain weight starts becoming constant is the point of GGD, and the linear phase of growth on the curve represents GGD.

4. Plant biomass

The plants were harvested at various growth stages and separated into stems and leaves, then dried in an oven at 80°C for four hours and then at 60°C till constant dry weight was recorded. Dry weights were recorded and expressed as g plant⁻¹.
Table 3.2: Temperature regimes during sowing, anthesis, 15DAA, 20 DAA and 25 DAA (From November 2012-March 2013)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Temperature</th>
<th>Sowing (°C)</th>
<th>Anthesis (°C)</th>
<th>15 DAA (°C)</th>
<th>20 DAA (°C)</th>
<th>25 DAA (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PBW 550</strong></td>
<td>Control</td>
<td>Max. 25</td>
<td>27.2</td>
<td>28</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>10.2</td>
<td>10.3</td>
<td>14.6</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>HT Stress</td>
<td>Max. 25</td>
<td>25.6</td>
<td>33.1</td>
<td>32.1</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>8.9</td>
<td>11.9</td>
<td>17.2</td>
<td>17.7</td>
</tr>
<tr>
<td><strong>RAJ 3765</strong></td>
<td>Control</td>
<td>Max. 25</td>
<td>25.5</td>
<td>29.5</td>
<td>31</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>12.4</td>
<td>15.7</td>
<td>16.2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>HT Stress</td>
<td>Max. 25</td>
<td>27.6</td>
<td>31.6</td>
<td>37.2</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>12.7</td>
<td>12.5</td>
<td>16.8</td>
<td>17.3</td>
</tr>
<tr>
<td><strong>KAUZ</strong></td>
<td>Control</td>
<td>Max. 25</td>
<td>27</td>
<td>29.7</td>
<td>34</td>
<td>32.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>9.2</td>
<td>16.4</td>
<td>19.4</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>HT Stress</td>
<td>Max. 25</td>
<td>26.1</td>
<td>30.6</td>
<td>35.6</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>11.7</td>
<td>13.3</td>
<td>19.9</td>
<td>15.9</td>
</tr>
<tr>
<td><strong>HD 2733</strong></td>
<td>Control</td>
<td>Max. 25</td>
<td>27</td>
<td>29.7</td>
<td>34</td>
<td>32.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>9.2</td>
<td>16.4</td>
<td>19.4</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>HT Stress</td>
<td>Max. 25</td>
<td>26.1</td>
<td>30.6</td>
<td>35.6</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>11.7</td>
<td>13.3</td>
<td>19.9</td>
<td>15.9</td>
</tr>
<tr>
<td><strong>WH 730</strong></td>
<td>Control</td>
<td>Max. 25</td>
<td>27.2</td>
<td>28</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>10.2</td>
<td>10.3</td>
<td>14.6</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>HT Stress</td>
<td>Max. 25</td>
<td>25.6</td>
<td>33.1</td>
<td>32.1</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min. 11</td>
<td>8.9</td>
<td>11.9</td>
<td>17.2</td>
<td>17.7</td>
</tr>
</tbody>
</table>

*HT Stress- High temperature stress  **DAA- Days after anthesis
Plate 3.2: Changes in maximum, minimum and mean temperature during grain development period of wheat genotypes.
5. Harvest index

Plants were collected at the time of the harvest. Grains were separated and weighed (economic yield). The whole plant dry weight was measured as biological yield. The harvest index was calculated as the ratio of the economic yield to biological yield and was expressed as percentage (Gardner et al., 1985).

\[ HI = \frac{\text{Economic yield}}{\text{Biological Yield}} \times 100 \]

6. Yield components

Following yield components were recorded at the time of final harvest:

- Grain yield per plant and 1000-grain weight (g).

3.4.2 Chemical analysis of grains

3.4.2.1. Estimation of sugars

Total soluble sugars were estimated by anthrone reagent method.

Preparation of reagents

- Sulphuric acid (95%): To 95.9 ml of sulphuric acid 4.1 ml of distilled water is added to make upto 100 ml.
- Anthrone reagent: Two hundred mg anthrone was dissolved in 100 ml of ice cold 95% sulphuric acid. Fresh reagent was prepared each time.
- Weighed amount of leaf samples were plunged in 95% ethanol and preserved.

There were three replications for each determination.

Extraction of sugars

For sugar analysis, the samples were boiled in 80% ethanol and the supernatant decanted into a beaker. The extraction was repeated four times (three times with 20 ml of 80% (v/v) ethanol in water and finally with 20 ml of distilled water) by boiling the sample for 4-5 min and decanting the supernatant (McCready et al., 1950). The combined sugar extract was made up to volume with distilled water in a 100 ml volumetric flask.
Clarification of the sugar extract

For clarification, 50 ml aliquot of the above sugar extract was evaporated in a water bath, taking care not to let the liquid dry out completely. Subsequently, the sample was treated with 1 ml saturated solution of lead acetate to precipitate the colloidal substances. It was then filtered into a 50 ml volumetric flask and made up to the volume. An aliquot of this solution was used for determining the total sugar by Anthrone reagent method (Sadasivam and Manickam, 1992).

Reagents used:

i. Arsenomolybadate reagent: The reagent was prepared by dissolving 25 g of ammonium molybdate in 450 mL distilled water and subsequently 21 mL of concentrated H$_2$SO$_4$ was added to it. A solution containing 3 g of sodium arsenate heptahydrate dissolved in 25 mL of distilled water was added to the above solution and mixed. The solution was incubated for 48 hours at 37ºC before it was ready for use.

ii. Alkaline copper tartarate (Somogyi’s) reagent: It was prepared by dissolving 24 g of anhydrous sodium carbonate and 12 g of sodium potassium tartarate in 250 mL of distilled water. 4 g Cupric sulphate pentahydrate solution was added to this while stirring continuously, followed by 16 g of sodium hydrogen carbonate. 180 g of sodium sulphate was added to 500 mL hot water and later boiled to expel air. After cooling, the above two solutions were mixed and volume was made to 1 litre.

Reducing sugars

One mL aliquot from the clarified extract was taken for the assay of reducing sugars. The volume was made to 2 mL with distilled water. One mL of Somogyi’s copper reagent was added and the final volume was made up to 10 mL. Absorbance of the blue coloured solution was measured at 530 nm. Standard curve was made using glucose in the range of 25-300 µg mL$^{-1}$. The stock solution of glucose was made by adding 100 mg glucose to 100 mL water. Working standards were prepared by diluting the stock solution with distilled water (100 µg mL$^{-1}$). The amount of reducing sugar was
calculated from standard curve of glucose and the results were expressed in terms of mg glucose equivalents g\(^{-1}\) DW.

**Determination of total sugars**

One ml of sugar sample was taken and to this 4 ml solution of anthrone regent was added. The mixture was heated on a boiling water bath for 8 min followed by cooling. The absorbance of green to dark green colour was read at 630 nm in UV-visible spectrophotometer (model Specord Bio-200, AnalytikJena, Germany). A blank and two freshly prepared glucose standards were also included with each set of samples.

**3.4.2.2. Estimation of starch content**

Starch content was also determined by Anthrone method (McCready *et al.*, 1950). The dry residue left after sugar extraction was powdered and 50 mg of it was hydrolyzed by boiling with 10 ml of 1N HCl for 30 minutes in a glycerine bath at 112-115 °C. After cooling, the samples were transferred into a 100 ml volumetric flask. The residue was repeatedly washed with distilled water until a negative test (iodine test) was obtained. The extract was collected and made to 100 ml. An aliquot of (0.5 ml) the above extract was made to 2.5 ml with distilled water. This was then mixed thoroughly with 10 ml of freshly prepared anthrone reagent (100 mg anthrone was dissolved in 100 ml chilled concentrated H\(_2\)SO\(_4\)), in a cold water bath. Subsequently, the tubes containing this mixture were kept in a boiling water bath for 15 minutes and then rapidly cooled in running tap water. Absorbance was measured at 620 nm. A reference standard curve was prepared using glucose in the range of 25-300µg. Starch content was calculated by multiplying the glucose values (1OD = 600 µg/g dw) with 0.9.

**3.4.2.3. Enzyme activities**

1. **Soluble starch synthase**

**Enzyme extraction**

The grain samples (1 g seed material) stored in liquid nitrogen were taken out and homogenized in a pre-chilled mortar and pestle with 5 ml of extraction buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl\(_2\), 1 mM DTT and 1 mg m\(^{-1}\) BSA. The homogenate was centrifuged at 30,000 g for 20 minutes at 4°C and the supernatant
saved for assay of the soluble enzyme. The pellet was washed twice with extraction buffer and re-centrifuged at 30,000 x g for 20 min at 4°C, and re-suspended in 3 ml of the same buffer to obtain granule bound starch synthase (George et al., 1994).

**Enzyme assay**

Soluble starch synthase activity was estimated by the amount of ADP formed from ADPG. ADP estimation was carried out by using, a preparation of pyruvate kinase, which catalyses the transfer of phosphate from phosphoenol pyruvate to ADP. Pyruvate liberated was estimated. Soluble starch synthase was assayed at 25°C for 30 minutes in a small test tube by adding 0.1 ml of enzyme extract to 0.15 ml of reaction mixture containing 25 µmol Tris-HCl buffer (pH 8.5), 0.2 µmol EDTA, 2.5 µmol glutathione, 5 µmol KCl, 0.5 mg glycogen and 0.25 µmol ADPG. The reaction was started by adding the enzyme and stopped later by immersing the tube in a boiling water bath for one minute and were then cooled immediately in a water bath to room temperature. Blank was run without the substrate ADPG. The amount of ADP liberated was then determined by the pyruvate kinase method (Leloir and Goldenberg 1960).

To estimate the ADP formed in the above reaction, 0.025 ml of phosphoenol pyruvate (0.01 M solution in 0.4 M KCl) and 0.025 ml of pyruvate kinase (8.4 U freshly diluted in 0.1 M MgSO₄) were added and incubated for 15 minutes at 37°C. At the end of the incubation period 0.15 ml of dinitrophenyl hydrazine (0.1% in 2N HCl) was added. After 5 minutes, 0.2 ml of 10 N NaOH and 1.1 ml of 95 per cent ethanol were added. The samples were mixed and centrifuged. The absorbance of the supernatant fluid was measured at 520 nm. The results were calculated from a standard curve drawn by using different concentrations (0-100 nmol) of pyruvate. The enzyme activity was expressed on per g fresh weight.

**3.4.2.3. Gene expression study for selected enzymes by RT-PCR.**

Nucleotide sequences for candidate genes were obtained from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to identify the homologs of candidate genes. For RT-PCR expression analysis the following oligonucleotide primers were designed manually, and oligo quality (to avoid primer dimmer, self dimer etc.), GC % and Tₘ were analysed by using Oligoanalyzer 3.0 tool
Integrated DNA Technologies, Coralville, IA 52241, USA).

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Length (bases)</th>
<th>GC %</th>
<th>Tm (ºC)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSSI-F</td>
<td>CAC AGA CAA GTG TCT CCC TCA TC</td>
<td>23</td>
<td>52.2</td>
<td>57.1</td>
<td>657</td>
</tr>
<tr>
<td>SSSI-R</td>
<td>TCG AAG ATC TGC TCG TAC TGC TC</td>
<td>23</td>
<td>52.2</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td>SSSI-F</td>
<td>CAT ACG GCA GAA CGA CTG GAA GAC</td>
<td>24</td>
<td>54.2</td>
<td>59.4</td>
<td>736</td>
</tr>
<tr>
<td>SSSI-R</td>
<td>CTT GAC GAG GAC GTC CTC GTA GAG</td>
<td>24</td>
<td>53.8</td>
<td>60.1</td>
<td></td>
</tr>
<tr>
<td>SSSIII-F</td>
<td>GTT GCC ATG CGT TAT GGA TCG ATC</td>
<td>24</td>
<td>50.0</td>
<td>58.6</td>
<td>283</td>
</tr>
<tr>
<td>SSSIII-R</td>
<td>GGT ACA ATT CAA TGT AGT CCA GTG</td>
<td>24</td>
<td>41.7</td>
<td>53.6</td>
<td></td>
</tr>
</tbody>
</table>

*Oligo concentration 1.0 μM, Na⁺ concentration 50 mM.

### 3.4.2.3.1. Isolation of total RNA from seed sample

Total RNA was also extracted from seed tissues of temperature stressed and control plants using RNAeasy kit (Qiagen Inc., Chatsworth CA 91311, USA, Cat No: 749040) according to the manufacturer’s instruction. But, seed endosperm contains very high levels of starch causing the solidification of samples in the guanidine isothiocyanate (GITC)-based RNA extraction buffers, such as TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and the RNeasy® kit (Qiagen, Valencia, CA, USA). To overcome this problem, preliminary extraction was done by modified method of Li and Trick (2005). About 50–100 mg seeds was ground to a fine powder in liquid nitrogen with pre-chilled mortar and pestle. The sample was then transferred into a pre-chilled 1.5-ml RNase-free microcentrifuge tube. A 400-μl extraction buffer I [100 mM Tris, pH 8.0, 150 mM LiCl, 50 mM EDTA, 1.5% sodium dodecyl sulfate (SDS), 1.5% 2-mercaptoethanol] was immediately added to the seed powder. After mixing the content with vigorous vortex mixing, 250 μl phenol: chloroform mixtures (1:1, pH 4.7) were added, and the samples were mixed well by inversion. Samples were then centrifuged immediately at 13,000× g for 15 min at 4ºC. The upper aqueous phase (around 250 μl)
was carefully transferred to a new 1.5 ml tube. Clear lysate was then passed through spin column and centrifuged at 8000g for 2 min in a bench top centrifuge at room temperature. The flow through was transferred to a new eppendorf tube and half volume of (225 μl) chilled ethanol (absolute) was added to clear lysate and mixed by pipetting. It was then passed through another spin column (pink column) and centrifuged for 1 min at room temperature at 8000 g. Flow through was discarded and 700 μl of RW1 buffer was added to the same column and centrifuged for 1 min. The same was repeated and centrifuged for 2 min. Finally RNA was eluted with 54 μl sterile RNAase free water by centrifuging at 8000 g for 1 min and was collected in an eppendorf. This was used as a template for reverse transcription polymerase chain reaction (RT-PCR).

### 3.4.2.3.2. RT-PCR expression analysis of target genes

Reverse transcriptase-polymerase chain reaction (RT-PCR) mixture using Qiagen One Step RT PCR Kit with gene specific and degenerate forward and reverse primers was prepared as per the protocol given below:

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity for 50 μl of reaction mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease free water</td>
<td>21.0 μl</td>
</tr>
<tr>
<td>5X RT buffer (Tris Cl, KCl, (NH₄)₂SO₄,</td>
<td>10.0 μl</td>
</tr>
<tr>
<td>13.5 mM MgCl₂, DTT; pH 8.7 at 20 °C)</td>
<td></td>
</tr>
<tr>
<td>dNTP Mix (10 mM each)</td>
<td>2.0 μl</td>
</tr>
<tr>
<td>Forward + Reverse primers (10 μM)</td>
<td>5.0 μl</td>
</tr>
<tr>
<td>RNA template (1 μg)</td>
<td>10.0 μl</td>
</tr>
<tr>
<td>RT Enzyme mix</td>
<td>2.0 μl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>50.0 μl</strong></td>
</tr>
</tbody>
</table>
The above reaction mixture was prepared in 0.2 ml PCR tubes and amplification carried out using QB 96 thermal cycler (Quanta Biotech, Byfleet, UK), with the following program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>30 min 50°C</td>
</tr>
<tr>
<td>Initial PCR activation step</td>
<td>15 min 95°C</td>
</tr>
</tbody>
</table>

3-step cycling

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature/Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturation</td>
<td>1 min 94°C</td>
</tr>
<tr>
<td>Annealing</td>
<td>1 min at different T_m for different genes</td>
</tr>
<tr>
<td>Extension</td>
<td>1 min 72°C</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>28</td>
</tr>
<tr>
<td>Final extension</td>
<td>10 min 72°C</td>
</tr>
</tbody>
</table>

Linear amplification for semi-quantitative RT-PCR was obtained with 27 cycles. To check the amplification, an aliquot of 5 μl of the reaction mixture was run on a 0.8 % (w/v) agarose gel stained with 0.5 μg ml⁻¹ ethidium bromide. 100 bp DNA ladder was included as a marker for size comparison of the amplified products. 1X TAE buffer was used to prepare the gel as well as for running buffer and electrophoresis was carried out at 5 V/cm (Sambrook et al., 1989). 6X loading dye was used to load the samples. The stained DNA products were photographed using Gel Documentation system.

3.5. Statistical analyses

The data was analysed statistically using 3 factorial CRD (Biochemical analysis and growth parameters) and CD at 5% and ANOVA were calculated. The analysis was done using OPSTAT programme available online on CCS, HISAR web site.
Association between the expression of soluble starch synthase family members and grain weight in wheat under high temperature stress

Neeraj Joshi, Ajay Arora, Rakesh Pandey
Division of Plant Physiology, Indian Agriculture Research Institute, New Delhi 110 012

4.1. Abstract
High temperature stress during the reproductive and grain filling phase is considered detrimental for wheat yield. In the present study, the relative sensitivity of five wheat genotypes viz. PBW 550, RAJ 3765, KAUZ, HD 2733 and WH 730 to high temperature stress during grain filling was assessed with respect to grain growth, activity of enzyme, soluble starch synthase (SSS), and transcription level variations of different isoforms of SSS. The objective of the study was to determine the relationship between starch accumulation and activity and gene expression SSS, under high temperature stress condition in contrasting wheat genotypes. High temperature stress decreased leaf area subsequently at different growth stages and biomass grain yield and at the time of harvest. Under high temperature stress grain growth rate (GGR) was also found to be significantly reduced, sugar increased and starch content decreased in all genotypes. However, under temperature stress KUAZ and WH 730 (relatively thermotolerant) showed lesser decrease in level of starch in comparison with other genotypes. The reduction in activity of soluble starch synthase (SSS) in KAUZ and WH 730 was lesser at 20 DAA when compared to 15 and 25 DAA suggested higher expression of some isoform during that period of growth in these genotypes. A comparatively higher reduction was observed in susceptible genotype HD 2733 at same stage of grain filling (20 DAA). Expression analysis of SSS family members at 20 DAA, revealed that in tolerant genotypes (KAUZ and WH 730) expression of isoforms SSSII and SSSIII were at higher levels as compared to susceptible genotype (HD 2733).

Key words: Soluble starch synthase, high temperature stress, grain-filling, wheat
4.2. Introduction

Under natural conditions plants are exposed to a variety of abiotic stresses, such as drought, high/low temperature, salinity and high light. Abiotic stresses are the primary cause of more than 50% crop losses worldwide. Among the abiotic stresses temperature stress is one of the severe environmental stresses. Wheat is the most important cereal crop of the world. Wheat cultivation in India started 5000 years ago (Feldman, 2001). It is a major crop of India and used as a staple food in the country and as per 2nd Advance Estimates, the production of wheat during the year 2012-13 is estimated at 92.30 million tonnes (Department of Agriculture and cooperation, Ministry of India). High temperature at the end of the season is a major determinant of wheat production under Indian conditions (Pandey et al., 2009). In general, a transient elevation in temperature, usually 10-15°C above ambient, is considered heat stress. The range of optimum temperatures for wheat growth is 18-24°C. Over these range increase in temperature even for a short period of 5-6 days short periods causes 20% or more yield losses in wheat (Stone and Nicolas, 1994). Globally high temperature stress affects 7 million hectares, while terminal heat stress affects almost 40 % of the irrigated wheat growing areas (Fisher and Byerlee, 1991). High temperature stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al., 2004). It has long been known that average wheat yield reduces by 4% for every one degree rise in ambient temperature during grain filling (McDonald et al., 1983). Even though optimum temperature for grain growth in wheat is 15°C (Chowdhury and Wardlaw, 1978), moderately high temperatures (25-32°C) for longer duration and very high temperatures (33-40°C) for a shorter period are commonly experienced in Mediterranean and subtropical environments, particularly during grain filling (Dhadhwal, 1989; Stone and Nicolas, 1994).

Starch is a major component of the grain and is the most important factor for yield while both the amount and composition of protein are critical for quality (Altenbach, 2012). Accumulation of starch is more sensitive than is the deposition of protein (Bhullar and Jenner, 1985), and it is the conversion of sucrose to starch within
the developing endosperm that is decreased by elevated temperature (Bhullar and Jenner, 1986). Under heat stress, crop photosynthesis declines and is unable to support both respiration and grain growth (Gent, 1994) and the relative importance of stored water soluble carbohydrates (WSC) to sustain grain growth becomes greater. Sustained periods of high temperature (30-38°C) from anthesis to maturity have been found to reduce grain yields mainly by reducing grain growth, but the magnitude of the effect can vary from 20 to 50% (Wardlaw et al., 1989a; Tewolde et al., 2006). High temperature during reproductive phase reduce fertility and seed yield in wheat (Saini et al., 1983).

On the sink side, grain filling is a process of active metabolism of carbohydrate and starch accumulation in kernels. It is generally accepted that four enzymes may play a key role in this process: Sucrose Synthase (SuSy), ADP glucose pyrophosphorylase (AGPase), starch synthase (SS), and starch branching enzyme (SBE) (Hawker and Jenner, 1993; Ahmadi and Baker, 2001; Hurkman et al., 2003). SSS activity is reported to be positively correlated with the rate of starch synthesis in wheat grains. Enzyme rate was found to have a temperature optimum between 20°C to 25°C Temperatures of more than 25°C adversely affect the activity of SSS in endosperm of wheat (Keeling et al., 1993).

The aim of the current study was to determine the differential response of soluble starch synthase family members of five wheat genotypes under high temperature stress condition.

4. 3. Materials and methods

4.3.1 Plant materials and treatments

Five wheat genotypes were taken for the experiment as suggested by the breeders on the basis of thermotolerance. The seeds were obtained from Division of Genetics, IARI, New Delhi.

1. PBW 550
2. Raj 3765
3. KAUZ
4. HD 2733
5. WH 730
Sowing was done in earthen pots (about 30 cm in diameter and 30 cm in depth) filled with clay loam soil and farmyard manure (FYM) in 3:1 ratio during *rabi* (winter) season.

### 4.3.2 Experimental treatment

The genotypes were raised in pot culture of Division of Plant Physiology, with date of sowing of 26\textsuperscript{th} November, 2012. Ten seeds per pot were taken at the time of sowing. Half of the pots from each genotype were shifted to polyhouse around 13\textsuperscript{th} February, 2013 (nearly a week prior to anthesis). In polyhouse the heat stress treatments were given with average of 32.4°C/15.5°C (difference of 2.6°C/1.5°C compared to ambient day/night temperature) at the time of grain filling (Table 3.2 and Plate 3.2).

### 4.3.3 Sample collection

Plants were sampled and observations were recorded for growth, physiological and biochemical parameters. Tagging was done from the date of anthesis observed. Grain samples were collected from control and high temperature stressed plants as per the tagging done, at 15 days after anthesis (DAA), and further at 5 days interval from first sampling (20 and 25). At each stage samples were collected in triplicate from three pots. For gene expression studies grain samples were used for total RNA extraction, which was done by storing the samples at -80°C.

### 4.3.4 Observations recorded

#### 4.3.4.1. Growth parameters

1. **Leaf area**

   All the green leaves from each replication (three plants per replication) were taken. Leaf area of flag leaf, main shoot leaves and total plant leaf area was measured using a standard leaf area meter (Model LiCOR 3100) and was expressed as cm\(^2\) plant\(^{-1}\).

2. **Grain growth duration (GGD)**

3. **Grain growth rate (GGR)**

   Grain growth was recorded by taking grains at 15 DAA and then at an interval of every 5 days up to 30 DAA. Grain growth curves were made using Microsoft Office Excel, 2007. The point on the curve where grain weight starts becoming constant is the point of GGD, and the linear phase of growth on the curve represents GGD.
4. Plant biomass

The plants were harvested at various growth stages and separated into stems and leaves then dried in an oven at 80°C for four hours and then at 60°C till constant dry weight was recorded. Dry weights were recorded and expressed as g plant$^{-1}$.

5. Harvest index

Plants were collected at the time of the harvest. Grains were separated and weighed (economic yield). The whole plant dry weight was measured as biological yield. The harvest index was calculated as the ratio of the economic yield to biological yield and was expressed as percentage (Gardner et al., 1985).

\[
\text{HI} = \frac{\text{Economic yield}}{\text{Biological Yield}} \times 100
\]

6. Yield components

Following yield components were recorded at the time of final harvest:

- Grain yield per plant and 1000-grain weight (g).

**4.3.4.2. Chemical analysis of grains**

**4.3.4 2.1. Estimation of sugars**

Total soluble sugars were estimated by anthrone reagent method.

**Extraction of sugars**

For sugar analysis, the samples were boiled in 80% ethanol and the supernatant decanted into a beaker. The extraction was repeated four times (three times with 20 ml of 80% (v/v) ethanol in water and finally with 20 ml of distilled water) by boiling the sample for 4-5 min and decanting the supernatant (McCready et al., 1950). The combined sugar extract was made up to volume with distilled water in a 100 ml volumetric flask.

**Clarification of the sugar extract**

For clarification, 50 ml aliquot of the above sugar extract was evaporated in a water bath, taking care not to let the liquid dry out completely. Subsequently, the sample was treated with 1 ml saturated solution of lead acetate to precipitate the colloidal substances. It was then filtered into a 50 ml volumetric flask and made up to the volume.
An aliquot of this solution was used for determining the total sugar by Anthrone reagent method (Sadasivam and Manickam, 1992).

**Determination of total sugars**

One ml of sugar sample was taken and to this 4 ml solution of anthrone regent was added. The mixture was heated on a boiling water bath for 8 min followed by cooling. The absorbance of green to dark green colour was read at 630 nm in UV-visible spectrophotometer (model Specord Bio-200, AnalytikJena, Germany). A blank and two freshly prepared glucose standards were also included with each set of samples.

**Reducing sugars**

Reducin sugars were estimated by Nelson-Somogyi method.

One mL aliquot from the clarified extract was taken for the assay of reducing sugars. The volume was made to 2 mL with distilled water. One mL of Somogyi’s copper reagent was added and the final volume was made up to 10 mL. Absorbance of the blue coloured solution was measured at 530 nm. Standard curve was made using glucose in the range of 25-300 µg mL⁻¹. The stock solution of glucose was made by adding 100 mg glucose to 100 mL water. Working standards were prepared by diluting the stock solution with distilled water (100 µg mL⁻¹). The amount of reducing sugar was calculated from standard curve of glucose and the results were expressed in terms of mg glucose equivalents g⁻¹ DW.

**4.3.4 2.2. Estimation of starch content**

Starch content was also determined by Anthrone method (McCready *et al.*, 1950). The dry residue left after sugar extraction was powdered and 50 mg of it was hydrolyzed by boiling with 10 ml of 1N HCl for 30 minutes in a glycerine bath at 112-115 °C. After cooling, the samples were transferred into a 100 ml volumetric flask. The residue was repeatedly washed with distilled water until a negative test (iodine test) was obtained. The extract was collected and made to 100 ml. An aliquot of (0.5 ml) the above extract was made to 2.5 ml with distilled water. This was then mixed thoroughly with 10 ml of freshly prepared anthrone reagent (100 mg anthrone was dissolved in 100 ml chilled concentrated H₂SO₄), in a cold water bath. Subsequently, the tubes containing this mixture were kept in a boiling water bath for 15 minutes and then rapidly cooled in
running tap water. Absorbance was measured at 620 nm. A reference standard curve was prepared using glucose in the range of 25-300 µg. Starch content was calculated by multiplying the glucose values (1OD = 600 µg/g dw) with 0.9.

**4.3.4.2.3. Enzyme activities**

1. **Soluble starch synthase**

**Enzyme extraction**

The grain samples (1 g seed material) stored in liquid nitrogen were taken out and homogenized in a pre-chilled mortar and pestle with 5 ml of extraction buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 1 mM DTT and 1 mg ml⁻¹ BSA. The homogenate was centrifuged at 30,000 g for 20 minutes at 4°C and the supernatant saved for assay of the soluble enzyme. The pellet was washed twice with extraction buffer and re-centrifuged at 30,000 x g for 20 min at 4°C, and re-suspended in 3 ml of the same buffer to obtain granule bound starch synthase (George et al., 1994).

**Enzyme assay**

Soluble starch synthase activity was estimated by the amount of ADP formed from ADPG. ADP estimation was carried out by using, a preparation of pyruvate kinase, which catalyses the transfer of phosphate from phosphoenol pyruvate to ADP. Pyruvate liberated was estimated. Soluble starch synthase was assayed at 25°C for 30 minutes in a small test tube by adding 0.1 ml of enzyme extract to 0.15 ml of reaction mixture containing 25 µmol Tris-HCl buffer (pH 8.5), 0.2 µmol EDTA, 2.5 µmol glutathione, 5 µmol KCl, 0.5 mg glycogen and 0.25 µmol ADPG. The reaction was started by adding the enzyme and stopped later by immersing the tube in a boiling water bath for one minute and were then cooled immediately in a water bath to room temperature. Blank was run without the substrate ADPG. The amount of ADP liberated was then determined by the pyruvate kinase method (Leloir and Goldenberg 1960).

To estimate the ADP formed in the above reaction, 0.025 ml of phosphoenol pyruvate (0.01 M solution in 0.4 M KCl) and 0.025 ml of pyruvate kinase (8.4 U freshly diluted in 0.1 M MgSO₄) were added and incubated for 15 minutes at 37°C. At the end of the incubation period 0.15 ml of dinitrophenyl hydrazine (0.1% in 2N HCl) was added. After 5 minutes, 0.2 ml of 10 N NaOH and 1.1 ml of 95 per cent ethanol were
added. The samples were mixed and centrifuged. The absorbance of the supernatant fluid was measured at 520 nm. The results were calculated from a standard curve drawn by using different concentrations (0-100 nmol) of pyruvate. The enzyme activity was expressed on per g fresh weight.

**4.3.4.3. Gene expression study for selected enzymes by RT-PCR.**

Nucleotide sequences for candidate genes were obtained from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used to identify the homologs of candidate genes. For RT-PCR expression analysis the following oligonucleotide primers were designed manually, and oligo quality (to avoid primer dimer, self dimer etc.), GC % and T_m were analysed by using Oligoanalyzer 3.0 tool (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/), Intergrated DNA Technologies, Coralville, IA 52241, USA).

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Length (bases)</th>
<th>GC %</th>
<th>Tm (°C)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSSI-F</td>
<td>CAC AGA CAA GTG TCT CCC TCA TC</td>
<td>23</td>
<td>52.2</td>
<td>57.1</td>
<td>657</td>
</tr>
<tr>
<td></td>
<td>TCG AAG ATC TGC TCG TAC TGC TC</td>
<td>23</td>
<td>52.2</td>
<td>58.3</td>
<td></td>
</tr>
<tr>
<td>SSSI-R</td>
<td>CAT ACG GCA GAA CGA CTG GAA GAC</td>
<td>24</td>
<td>54.2</td>
<td>59.4</td>
<td>736</td>
</tr>
<tr>
<td></td>
<td>CTT GAC GAG GAC GTC CTC GTA GAG</td>
<td>24</td>
<td>53.8</td>
<td>60.1</td>
<td></td>
</tr>
<tr>
<td>SSSII-F</td>
<td>GTT GCC ATG CGT TAT GGA TCG ATC</td>
<td>24</td>
<td>50.0</td>
<td>58.6</td>
<td>283</td>
</tr>
<tr>
<td>SSSII-R</td>
<td>GGT ACA ATT CAA TGT AGT CCA GTG</td>
<td>24</td>
<td>41.7</td>
<td>53.6</td>
<td></td>
</tr>
</tbody>
</table>

*Oligo concentration 1.0 μM, Na⁺ concentration 50 mM.

**4.3.4.3.1. Isolation of total RNA from seed sample**
Total RNA was also extracted from seed tissues of temperature stressed and control plants using RNAeasy kit (Qiagen Inc., Chatsworth CA 91311, USA, Cat No: 749040) according to the manufacturer’s instruction. But, seed endosperm contained very high levels of starch causing the solidification of samples in the guanidine isothiocyanate (GITC)-based RNA extraction buffers, such as TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) and the RNeasy® kit (Qiagen, Valencia, CA, USA). To overcome this problem, preliminary extraction was done by modified method of Li and Trick (2005). About 50–100 mg seeds were ground to a fine powder in liquid nitrogen with pre-chilled mortar and pestle. The sample was then transferred into a pre-chilled 1.5-ml RNase-free microcentrifuge tube. A 400-μl extraction buffer I [100 mM Tris, pH 8.0, 150 mM LiCl, 50 mM EDTA, 1.5% sodium dodecyl sulfate (SDS), 1.5% 2-mercaptoethanol] was immediately added to the seed powder. After mixing the content with vigorous vortex mixing, 250 μl phenol: chloroform mixtures (1:1, pH 4.7) were added, and the samples were mixed well by inversion. Samples were then centrifuged immediately at 13,000× g for 15 min at 4°C. The upper aqueous phase (around 250 μl) was carefully transferred to a new 1.5 ml tube. Clear lysate was then passed through spin column and centrifuged at 8000g for 2 min in a bench top centrifuge at room temperature. The flow through was transferred to a new eppendorf tube and half volume of (225 μl) chilled ethanol (absolute) was added to clear lysate and mixed by pipetting. It was then passed through another spin column (pink column) and centrifuged for 1 min at room temperature at 8000 g. Flow through was discarded and 700 μl of RW1 buffer was added to the same column and centrifuged for 1 min. The same was repeated and centrifuged for 2 min. Finally RNA was eluted with 54 μl sterile RNAase free water by centrifuging at 8000 g for 1 min and was collected in an eppendorf. This was used as a template for reverse transcription polymerase chain reaction (RT-PCR).

4.3.4.3.2. RT-PCR expression analysis of target genes

Reverse transcriptase-polymerase chain reaction (RT-PCR) mixture using Qiagen One Step RT PCR Kit with gene specific and degenerate forward and reverse primers was prepared as per the protocol given below:
Reagents | Quantity for 50 μl of reaction mixture
---|---
Nuclease free water | 21.0 μl
5X RT buffer (Tris Cl, KCl, (NH₄)₂SO₄, 13.5 mM MgCl₂, DTT; pH 8.7 at 20 ºC) | 10.0 μl
dNTP Mix (10 mM each) | 2.0 μl
Forward + Reverse primers (10 μM) | 5.0 μl
RNA template (1 μg) | 10.0 μl
RT Enzyme mix | 2.0 μl
Total volume | 50.0 μl

The above reaction mixture was prepared in 0.2 ml PCR tubes and amplification carried out using *QB 96* thermal cycler (*Quanta Biotech, Byfleet, UK*), with the following program:

Reverse Transcription | 30 min 50°C
Initial PCR activation step | 15 min 95°C

**3-step cycling**

Denaturation | 1 min 94°C
Annealing | 1 min at different T_m for different genes
Extension | 1 min 72°C
Number of cycles | 28
Final extension | 10 min 72°C
Linear amplification for semi-quantitative RT-PCR was obtained with 27 cycles. To check the amplification, an aliquot of 5 μl of the reaction mixture was run on a 0.8 % (w/v) agarose gel stained with 0.5 μg ml\(^{-1}\) ethidium bromide. 100 bp DNA ladder was included as a marker for size comparison of the amplified products. 1X TAE buffer was used to prepare the gel as well as for running buffer and electrophoresis was carried out at 5 V/cm (Sambrook \textit{et al.}, 1989). 6X loading dye was used to load the samples. The stained DNA products were photographed using Gel Documentation system.

\subsection*{4.3.4. Statistical analyses}

The data was analysed statistically using 3 factorial CRD (Biochemical analysis and growth parameters) and CD at 5\% and ANOVA were calculated. The analysis was done using OPSTAT programme available online on CCS, HISAR web site.

\section*{4.4. Results}

\subsection*{4.4.1. Leaf area per plant}

Results on the effect of high temperature stress on leaf area per plant of wheat genotypes are reported in Table 4.4.1 and Fig 4.4.1. Leaf area per plant showed high temperature stress induced reductions in all the genotypes. Mean reduction in leaf area per plant under high temperature stress at 15, 20 and 25 DAA stages were 17\%, 24\% and 26\%, respectively. HD 2733 showed lowest reduction in leaf area of 6.5\% at 20 DAA and WH 730 showed 9.6\% decline at 25 DAA, whereas KAUZ showed highest reduction in total leaf area 44.5\% at 20 DAA and RAJ 3765 showed reduction of 51\% at 25 DAA.

None of the combinations between stages, treatments and genotypes showed significance differences. However, significance difference is observed between stages, genotypes and treatments, when observed individually.

\subsection*{4.4.2. Total sugar content}

Results on the effect of high temperature stress on total sugar content in the grains of wheat genotypes are presented in Table 4.2.2 and Fig 4.2.2. Total sugar contents increased significantly under all high temperature stress treatments at all the stages studied, irrespective of the genotypes. The sugar content was found to be highest at stage of 15 DAA. The increases were considerably higher in tolerant genotype
Table 4.4.1: Effect of high temperature stress on leaf area per plant (cm\(^2\)) in wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature Stress</td>
<td>Mean</td>
<td>% Change</td>
<td>Control</td>
</tr>
<tr>
<td>PBW 550</td>
<td>412.0</td>
<td>389.0</td>
<td>-11.2</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>457.0</td>
<td>416.8</td>
<td>-17.6</td>
</tr>
<tr>
<td>KAUZ</td>
<td>433.5</td>
<td>384.8</td>
<td>-22.5</td>
</tr>
<tr>
<td>HD 2733</td>
<td>432.0</td>
<td>406.3</td>
<td>-11.9</td>
</tr>
<tr>
<td>WH 730</td>
<td>431.9</td>
<td>395.4</td>
<td>286.2</td>
</tr>
</tbody>
</table>

Factors

<table>
<thead>
<tr>
<th>C.D.</th>
<th>Stages (S)</th>
<th>Genotypes (G)</th>
<th>S X G</th>
<th>Treatments (T)</th>
<th>S X T</th>
<th>G X T</th>
<th>S X G X T</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.839</td>
<td>N/A</td>
<td>42.125</td>
<td>15.382</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
Fig 4.4.1: Effect of high temperature stress on leaf area per plant (cm$^2$) in wheat genotypes at different growth stages.
KAUZ, followed by WH 730 at 15 DAA. PBW 550 showed lowest high temperature stress induced accumulation of total sugars. Same pattern for lowest total sugar accumulation was there for PBW 550, and highest for HD 2733 under high temperature stress condition at 20 DAA. Sugar accumulation was found to be highest at 15 DAA and significantly decreased at 25 DAA in all the genotypes. At 25 DAA less percent increase in total sugar accumulation in relatively tolerant genotypes (RAJ 3765, KAUZ and WH 730) that ranges from 8% to 13%. At 15 DAA, nearly two times more total sugar content was present as in comparison to 25 DAA.

Significant differences were observed between stages, genotypes and treatments. But the interaction between stages and treatments, however, interaction between stages, treatments and genotypes showed no significant difference.

### 4.4.3. Reducing sugar content

Results on the effect of high temperature stress on reducing sugar content in the grains of wheat genotypes are presented in Table 4.3.3 and Fig 4.3.3. Reducing sugar levels decreased significantly under temperature stress condition at all the stages of wheat grain studied (15 DAA, 20 DAA and 25 DAA), and in all the genotypes, irrespective of treatments. However, high reducing sugar content was observed in temperature stress treated plants as compared to control plants. The reducing sugar content was found to be highest at 15 DAA stage of the plant. Wheat genotype RAJ 3765 showed highest percent of stress induced accumulation of reducing sugars, about 20% and percent increase in PBW 550 and WH 730 was 16% to 17%, respectively, at 15 DAA. However, HD 2733, showed a decline of 3.8% in reducing sugar accumulation at 15 DAA under high temperature stress. Across all the stages and stress treatments, average reducing sugar content in the grains was highest in RAJ 3765 and WH 730.

Significant differences were observed between stages, genotypes, treatments and interaction between stages and genotypes. No other interactions showed significant differences.

### 4.4.4. Non-Reducing sugar content

Results on the effect of high temperature stress on non-reducing sugar content in the grains of wheat genotypes are presented in Table 4.4.4 and Fig 4.4.4. High non-
### Table 4.2.2: Effect of high temperature stress on total sugars (mg g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Days after anthesis</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBW 550</td>
<td></td>
<td>79.4</td>
<td>91.5</td>
<td>85.4</td>
<td>15.2</td>
<td>55.5</td>
<td>69.3</td>
<td>62.4</td>
<td>24.9</td>
<td>35.6</td>
<td>53.6</td>
<td>44.6</td>
<td>50.6</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td></td>
<td>75.8</td>
<td>93.3</td>
<td>84.6</td>
<td>23.0</td>
<td>67.6</td>
<td>75.5</td>
<td>71.5</td>
<td>11.7</td>
<td>51.4</td>
<td>56.9</td>
<td>54.2</td>
<td>10.6</td>
</tr>
<tr>
<td>KAUZ</td>
<td></td>
<td>93.0</td>
<td>103.0</td>
<td>98.0</td>
<td>10.8</td>
<td>69.3</td>
<td>71.6</td>
<td>70.5</td>
<td>3.3</td>
<td>44.1</td>
<td>49.6</td>
<td>46.9</td>
<td>12.5</td>
</tr>
<tr>
<td>HD 2733</td>
<td></td>
<td>82.8</td>
<td>98.9</td>
<td>90.8</td>
<td>19.5</td>
<td>63.7</td>
<td>82.3</td>
<td>73.0</td>
<td>29.1</td>
<td>45.6</td>
<td>63.9</td>
<td>54.8</td>
<td>40.3</td>
</tr>
<tr>
<td>WH 730</td>
<td></td>
<td>80.4</td>
<td>97.9</td>
<td>89.2</td>
<td>21.8</td>
<td>60.4</td>
<td>73.7</td>
<td>67.0</td>
<td>22.1</td>
<td>38.9</td>
<td>42.3</td>
<td>40.6</td>
<td>8.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>82.3</td>
<td>96.9</td>
<td>89.6</td>
<td></td>
<td>63.3</td>
<td>74.5</td>
<td>68.9</td>
<td></td>
<td>43.1</td>
<td>53.3</td>
<td>48.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stages (S)</th>
<th>Genotypes (G)</th>
<th>S X G</th>
<th>Treatments (T)</th>
<th>S X T</th>
<th>G X T</th>
<th>S X G X T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>39.696</td>
<td>51.247</td>
<td>88.762</td>
<td>32.411</td>
<td>N/A</td>
<td>72.474</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Fig 4.4.2: Effect of high temperature stress on total sugar content (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages.
reducing sugar content was observed in temperature stress treated plants as compared to control plants, at all the stages (15, 20 and 25 DAA) of wheat development studied, irrespective of genotypes. HD 2733 showed average maximum percent increase throughout the stages studied. However, a decline on percent accumulation of 6.3% to 6.8% was observed in WH 730 and RAJ 3765, respectively, at 25 DAA.

Same trend of significance differences observed, as in case of reducing sugars.

4.4.5. Starch content

Results on the effect of temperature stress on starch content at different stages (15, 20 and 25 DAA) in wheat grains of different genotypes are presented in Table 4.4.5 and Fig 4.4.5. In contrast to sugar content, starch content decreased with the high temperature stress treatment in all the five genotypes at all the stages studied. At 15 DAA HD 2733 and RAJ 3765 showed 30 and 36% decreases, respectively, in grain starch content under high temperature stress. PBW 550, WH 730 and KAUZ showed lesser decrease in starch content, ranging from 18, 26 and 27%, respectively, under high temperature stress at 15 DAA. At 25 and 30 DAA percent decline in grain starch content under high temperature stress, ranging from 9 to 15% compared to control. KAUZ, HD 2733 and WH 730 showed lesser decrease in starch content under high temperature stress treatment at 20 and 25 DAA. HD 2733 maintained lowest decline of 9.3 and 9.1% in starch content, both at 20 and 25 DAA, respectively, under high temperature stress.

Significant differences were there again between stages, genotypes and treatments, as in total sugar content. Except the interaction between stages and genotypes, none of the interaction was significant.

4.4.6. Soluble starch synthase activity

The results on the effect of high temperature stress treatment on the soluble starch synthase (SSS) activity in the grains of wheat genotypes are presented in Table 4.4.6 and Fig 4.4.6. Soluble starch synthase activity decreased under high temperature stress in all the genotypes at all the stages studied (15, 20 and 25 DAA). Genotypes WH 730 and PBW 550 showed lesser decreases in SSS activity, ranging from 7-14% and RAJ 3765, KAUZ and HD 2733 from 33-35% under high temperature stress at 15 DAA. Wheat genotype RAJ 3765 showed highest stress induced decrease of 22% in starch
Table 4.3.3: Effect of high temperature stress on reducing sugars (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Days after anthesis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Temperature Stress</td>
<td>Mean</td>
<td>% Change</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>PBW 550</td>
<td>52.6</td>
<td>61.5</td>
<td>57.0</td>
<td>16.8</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>35.4</td>
<td>42.5</td>
<td>38.9</td>
<td>20.1</td>
</tr>
<tr>
<td>KAUZ</td>
<td>53.1</td>
<td>55.8</td>
<td>54.5</td>
<td>5.0</td>
</tr>
<tr>
<td>HD 2733</td>
<td>53.3</td>
<td>51.2</td>
<td>52.2</td>
<td>-3.8</td>
</tr>
<tr>
<td>WH 730</td>
<td>45.2</td>
<td>52.7</td>
<td>48.9</td>
<td>16.5</td>
</tr>
<tr>
<td>Mean</td>
<td>47.9</td>
<td>52.7</td>
<td>50.3</td>
<td>34.1</td>
</tr>
</tbody>
</table>

Factors: Stages (S), Genotypes (G), S X G, Treatments (T), S X T, G X T, S X G X T

C.D. 1.909 2.464 4.268 1.558 N/A N/A N/A
Fig 4.3.3: Effect of high temperature stress on reducing sugar content (mg g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages
Table 4.4.4: Effect of high temperature stress on non-reducing sugars (mg g⁻¹ dry weight) in grains of wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Days after anthesis</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Temperature Stress</td>
<td>Mean</td>
<td>% Change</td>
</tr>
<tr>
<td>PBW 550</td>
<td>26.8</td>
<td>30.0</td>
<td>28.4</td>
<td>12.0</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>40.5</td>
<td>50.8</td>
<td>45.6</td>
<td>25.7</td>
</tr>
<tr>
<td>KAUZ</td>
<td>39.9</td>
<td>47.2</td>
<td>43.5</td>
<td>18.4</td>
</tr>
<tr>
<td>HD 2733</td>
<td>29.5</td>
<td>47.7</td>
<td>38.6</td>
<td>61.5</td>
</tr>
<tr>
<td>WH 730</td>
<td>35.2</td>
<td>45.3</td>
<td>40.2</td>
<td>28.6</td>
</tr>
<tr>
<td>Mean</td>
<td>34.4</td>
<td>44.2</td>
<td>39.3</td>
<td>29.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stages (S)</th>
<th>Genotypes (G)</th>
<th>Treatments (T)</th>
<th>S X T</th>
<th>G X T</th>
<th>S X G X T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>3.844</td>
<td>4.962</td>
<td>8.595</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Fig 4.4.4: Effect of high temperature stress on non-reducing sugar content (mg g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages.
Table 4.4.5: Effect of high temperature stress on total starch (mg g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>15 Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>120.4</td>
<td>98.4</td>
<td>109.4</td>
<td>-18.3</td>
<td>163.6</td>
<td>139.1</td>
<td>151.3</td>
<td>-15.0</td>
<td>216.7</td>
<td>182.8</td>
<td>199.8</td>
<td>-15.7</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>167.9</td>
<td>106.9</td>
<td>137.4</td>
<td>-36.3</td>
<td>173.2</td>
<td>133.9</td>
<td>153.5</td>
<td>-22.7</td>
<td>208.7</td>
<td>167.6</td>
<td>188.2</td>
<td>-19.7</td>
</tr>
<tr>
<td>KAUZ</td>
<td>107.9</td>
<td>78.1</td>
<td>93.0</td>
<td>-27.6</td>
<td>152.1</td>
<td>133.2</td>
<td>142.7</td>
<td>-12.4</td>
<td>192.2</td>
<td>162.7</td>
<td>177.5</td>
<td>-15.4</td>
</tr>
<tr>
<td>HD 2733</td>
<td>130.9</td>
<td>91.3</td>
<td>111.1</td>
<td>-30.2</td>
<td>185.6</td>
<td>168.4</td>
<td>177.0</td>
<td>-9.3</td>
<td>210.2</td>
<td>191.0</td>
<td>200.6</td>
<td>-9.1</td>
</tr>
<tr>
<td>WH 730</td>
<td>176.8</td>
<td>129.3</td>
<td>153.0</td>
<td>-26.9</td>
<td>198.1</td>
<td>173.4</td>
<td>185.7</td>
<td>-12.5</td>
<td>222.6</td>
<td>198.7</td>
<td>210.7</td>
<td>-10.7</td>
</tr>
<tr>
<td>Mean</td>
<td>140.8</td>
<td>100.8</td>
<td>120.8</td>
<td></td>
<td>174.5</td>
<td>149.6</td>
<td>162.0</td>
<td></td>
<td>210.1</td>
<td>180.6</td>
<td>195.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stages (S)</th>
<th>Genotypes (G)</th>
<th>S X G</th>
<th>Treatments (T)</th>
<th>S X T</th>
<th>G X T</th>
<th>S X G X T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>7.275</td>
<td>9.392</td>
<td>16.267</td>
<td>5.94</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Fig 4.4.5: Effect of high temperature stress on starch content (mg g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages.
Table 4.4.6: Effect of high temperature stress on soluble starch synthase activity (nmol min\(^{-1}\) g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Days after anthesis</th>
<th>Days after anthesis</th>
<th>Days after anthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Temperature Stress</td>
<td>Mean</td>
</tr>
<tr>
<td>PBW 550</td>
<td>15</td>
<td>280.9</td>
<td>239.5</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>20</td>
<td>368.5</td>
<td>246.3</td>
</tr>
<tr>
<td>KAUZ</td>
<td>25</td>
<td>319.2</td>
<td>207.1</td>
</tr>
<tr>
<td>HD 2733</td>
<td></td>
<td>241.5</td>
<td>156.2</td>
</tr>
<tr>
<td>WH 730</td>
<td></td>
<td>219.3</td>
<td>204.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>285.9</td>
<td>210.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors</th>
<th>Stages (S)</th>
<th>Genotypes (G)</th>
<th>S X G</th>
<th>Treatments (T)</th>
<th>S X T</th>
<th>G X T</th>
<th>S X G X T</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>13.238</td>
<td>17.09</td>
<td>29.601</td>
<td>10.809</td>
<td>N/A</td>
<td>24.169</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Fig 4.4.6: Effect of high temperature stress on soluble starch synthase activity (nmol min\(^{-1}\)g\(^{-1}\) dry weight) in grains of wheat genotypes at different growth stages.
content and 35%, in SSS activity at 20 DAA. In case of WH 730 the decrease was 7, 14 and 10% at 15, 20 and 25 DAA, respectively. HD 2733 and KAUZ showed nearly 35% decrease in SSS activity under high temperature stress at 15 DAA. RAJ 3765 showed maximum decline of 35% and 38% at 20 and 25 DAA, respectively under high temperature stress.

Significance differences were observed between stages, genotypes and treatments. But no significant differences occurred in interaction of all the three, i.e., between stages, genotypes and treatments. Same case of non-significance observed with interaction in between stages and treatments.

4.4.7. Soluble starch synthase expressions

4.4.7.1 Soluble starch synthase I (SSSI)

The results on the effect of high temperature stress treatment on soluble starch synthase I (SSSI) gene expression in the grains of wheat genotypes are presented in Plate 4.4.7.1. In case of SSSI gene, RT-PCR was performed with gene specific primers and expected amplicons of size 657 bp were obtained in all the five genotypes in both the treatment and control and at two stages studied (15 DAA and 20 DAA). Expression of SSSI decreased under high temperature stress in grains of all the five genotypes compared to control. High temperature stress induced decline in gene expression was observed in all the five genotypes, however, very less decline were observed in genotypes WH 730 and HD 2733 at 15 DAA and in genotypes RAJ 3765 and KAUZ at 20 DAA. PBW 550, HD 2733 and WH 730 showed highest percent of decline in SSSI gene expression under high temperature stress at 20 DAA as compared to other genotypes.

4.4.7.2. Soluble starch synthase II (SSSII)

The results on the effect of high temperature stress treatment on soluble starch synthase II (SSSII) gene expression in the grains of wheat genotypes are presented in Plate 4.4.7.2. In case of SSSII gene, RT-PCR was performed with gene specific primers and expected amplicons of size 736 bp were obtained in all the five genotypes in both the treatment and control at 15 DAA and 20 DAA. High temperature stress induced decline in SSSII gene expression was comparatively less observed than for SSSI and
Plate 4.4.7.1: RT-PCR expression analysis of *SSSI* gene in grain samples (M- 100 bp DNA ladder, LANE 1, 3, 5, 7, 9—CONTROL of genotype PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730, LANE 2, 4, 6, 8, 10—HIGH TEMPERATURE STRESS of PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730)
Plate 4.4.7.2: RT-PCR expression analysis of SSSII gene in grain samples (M- 100 bp DNA ladder, LANE 1, 3, 5, 7, 9-CONTROL of genotype PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730, LANE 2, 4, 6, 8, 10–HIGH TEMPERATURE STRESS of PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730)
Plate 4.4.7.3: RT-PCR expression analysis of SSSIII gene in grain samples (M- 100 bp DNA ladder, LANE 1, 3, 5, 7, 9-CONTROL of genotype PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730, LANE 2, 4, 6, 8, 10–HIGH TEMPERATURE STRESS of PBW 550, RAJ 3765, KAUZ, HD 2733, WH 730)
SSSIII isoforms in all the five genotypes studied. High temperature stress induced down regulation in SSSI expression was observed in all the five genotypes. However, very little decline in gene expression was observed in high temperature stressed grains of the tolerant genotypes KAUZ and WH 730 at both the stages studied while greater declines in expression were observed in genotypes PBW 550 and HD 2733 at both the stages (15 and 20 DAA). Very little expression of SSSI was observed in case of RAJ 3765 under both control and high temperature stress condition at 15 DAA. Gene expression in case of KAUZ was more than WH 730 under high temperature stress condition at 20 DAA.

**4.4.7.3. Soluble starch synthase III (SSSIII)**

The results on the effect of high temperature stress treatment on soluble starch synthase III (SSSIII) gene expression in the grains of wheat genotypes are presented in Plate 4.4.7.3. In case of SSSIII gene, RT-PCR was performed with gene specific primers and expected amplicons of size 283 bp were obtained in all the five genotypes in both the treatment and control at 15 DAA and 20 DAA. Lesser decline in gene expression was observed in all the genotypes under high temperature stress treatment at both the stages, as compared to controls of the respective genotypes, except RAJ 3765 and HD 2733 which showed comparatively greater high temperature stress induced down regulation at 20 DAA.

**4.4.8. Gain growth rate (GGR) (mg/day)**

Results on the effect of high temperature stress on GGR in the grains of wheat genotypes are presented in Table 4.4.8 and Fig 4.4.8. The GGR was found to be less in WH under high temperature stress treatment. The decrease was considerably lower in PBW 550 of about 5.7%, followed by RAJ 3765 and HD 2733. Wheat genotype KAUZ showed highest high temperature stress induced decrease in GGR as compared to other genotypes.

Significant differences were observed between genotypes and treatments. However, the interaction between stages and showed no significant difference.

**4.4.9. Grain yield per plant**

Results on the effect of high temperature stress on grain yield per plant are reported in Table 4.4.9 and Fig 4.4.9. The mean reduction in grain yield under
Table 4.4.9: Effect of high temperature stress on grain yield per plant (%) in wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>16.1</td>
<td>12.0</td>
<td>14.0</td>
<td>-25.3</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>18.4</td>
<td>11.9</td>
<td>15.1</td>
<td>-35.6</td>
</tr>
<tr>
<td>KAUZ</td>
<td>18.1</td>
<td>14.2</td>
<td>16.2</td>
<td>-21.5</td>
</tr>
<tr>
<td>HD 2733</td>
<td>18.4</td>
<td>15.2</td>
<td>16.8</td>
<td>-17.1</td>
</tr>
<tr>
<td>WH 730</td>
<td>21.3</td>
<td>15.0</td>
<td>18.1</td>
<td>-29.6</td>
</tr>
<tr>
<td>Mean</td>
<td>18.4</td>
<td>13.7</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

Factors

<table>
<thead>
<tr>
<th></th>
<th>Genotypes (A)</th>
<th>Treatments (B)</th>
<th>(A X B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>2.117</td>
<td>1.339</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Fig 4.4.9: Effect of high temperature stress on grain yield per plant (%) in wheat genotypes.
Table 4.4.8: Effect of high temperature stress on grain growth rate (GRR) (mg per day) in wheat genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>3.4</td>
<td>3.2</td>
<td>3.3</td>
<td>-5.7</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>3.7</td>
<td>3.3</td>
<td>3.5</td>
<td>-10.4</td>
</tr>
<tr>
<td>KAUZ</td>
<td>3.3</td>
<td>2.7</td>
<td>3.0</td>
<td>-16.8</td>
</tr>
<tr>
<td>HD 2733</td>
<td>4.0</td>
<td>3.4</td>
<td>3.7</td>
<td>-14.3</td>
</tr>
<tr>
<td>WH 730</td>
<td>3.8</td>
<td>3.4</td>
<td>3.6</td>
<td>-11.5</td>
</tr>
<tr>
<td>Mean</td>
<td>3.6</td>
<td>3.2</td>
<td>3.4</td>
<td></td>
</tr>
</tbody>
</table>

Factors

<table>
<thead>
<tr>
<th></th>
<th>Genotypes (A)</th>
<th>Treatments (B)</th>
<th>(A X B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D.</td>
<td>0.399</td>
<td>0.252</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Fig 4.4.8: Effect of high temperature stress on grain growth rate (GRR) (mg per day) in wheat genotypes.
temperature stress was 25.8% over control. However, HD 2733 showed least reduction of 17.1%, while the highest yield reduction under high temperature stress condition was observed in RAJ 3765, which showed around 38% reduction in comparison to control.

Significant differences were showed by genotypes and treatments alone, though interaction between genotypes and treatments showed no significant differences.

4.4.10. 1000-grain weight

Results on 1000 grain weight (test weight) of wheat genotypes under high temperature stress condition are reported in Table 4.4.10 and Fig 4.4.10. Test weight of seeds decreased significantly under high temperature stress in all the genotypes. Genotypes KAUZ, PBW 550, RAJ 3765 and WH 730 showed lesser reductions in test weight, which varied from 4% to 7.2% while in case of HD 2733, it was 12.5%.

4.4.11. Total biomass per plant

Results on the effect of high temperature stress on total dry matter production in wheat genotypes are shown in Table 4.4.11 and Fig 4.4.11. Temperature stress drastically reduced the total dry matter accumulation in all the genotypes with overall reduction of 18.4%. However, the reductions were much higher in genotypes KAUZ, RAJ 3765 and WH 730, which varied from 21% to 28%. In case of genotypes HD 2733 and PBW 550 the reductions in biomass ranged from 6% to 12%.

There were significant differences between treatments and genotypes, however, no significant difference was observed in interaction between the genotypes and treatments.

4.4.12. Harvest index

Results on the effect of high temperature stress on the harvest index of wheat genotypes are shown in Table 4.4.12 and Fig 4.4.12. Mean reduction in harvest index was about 7.3% affected by high temperature increase. In case of genotypes KAUZ minimum reduction was observed, 0.7%. However, in genotype PBW 550 yield reductions was 15%, which was highest compared to other genotypes studied.

Significant differences were observed between genotypes and treatments, however, interaction between genotype and treatments showed no significant difference.
Table 4.4.11: Effect of high temperature stress on total biomass per plant (g) in wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>33.1</td>
<td>29.1</td>
<td>31.1</td>
<td>-12.0</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>46.4</td>
<td>33.1</td>
<td>39.7</td>
<td>-28.7</td>
</tr>
<tr>
<td>KAUZ</td>
<td>43.9</td>
<td>34.4</td>
<td>39.1</td>
<td>-21.6</td>
</tr>
<tr>
<td>HD 2733</td>
<td>44.5</td>
<td>41.7</td>
<td>43.1</td>
<td>-6.2</td>
</tr>
<tr>
<td>WH 730</td>
<td>48.0</td>
<td>36.4</td>
<td>42.2</td>
<td>-24.1</td>
</tr>
<tr>
<td>Mean</td>
<td>43.2</td>
<td>34.9</td>
<td>39.1</td>
<td></td>
</tr>
</tbody>
</table>

Factors | Genotypes (A) | Treatments (B) | (A X B) |
---------|----------------|----------------|---------|
C.D.     | 5.389          | 3.408          | N/A     |

Fig 4.4.11: Effect of high temperature stress on total biomass per plant (g) in wheat genotypes.
Table 4.4.10: Effect of high temperature stress on test weight per Plant (g) of wheat genotypes at the time of harvesting

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>44.0</td>
<td>40.8</td>
<td>42.4</td>
<td>-7.2</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>44.2</td>
<td>41.9</td>
<td>43.1</td>
<td>-5.2</td>
</tr>
<tr>
<td>KAUZ</td>
<td>45.6</td>
<td>43.8</td>
<td>44.7</td>
<td>-4.0</td>
</tr>
<tr>
<td>HD 2733</td>
<td>47.1</td>
<td>41.2</td>
<td>44.1</td>
<td>-12.5</td>
</tr>
<tr>
<td>WH 730</td>
<td>43.7</td>
<td>41.1</td>
<td>42.4</td>
<td>-5.8</td>
</tr>
<tr>
<td>Mean</td>
<td>44.9</td>
<td>41.8</td>
<td>43.3</td>
<td></td>
</tr>
</tbody>
</table>

Factors | Genotypes (A) | Treatments (B) | (A X B) |
---------|----------------|----------------|---------|
C.D.     | N/A            | N/A            | 2.198   |

Fig 4.4.10: Effect of high temperature stress on test weight per Plant (g) of wheat genotypes at the time of harvesting
Table 4.4.12: Effect of high temperature stress on harvest index per plant (%) in wheat genotypes.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control</th>
<th>Temperature Stress</th>
<th>Mean</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBW 550</td>
<td>48.4</td>
<td>41.2</td>
<td>44.8</td>
<td>-15.0</td>
</tr>
<tr>
<td>RAJ 3765</td>
<td>39.6</td>
<td>35.9</td>
<td>37.8</td>
<td>-9.3</td>
</tr>
<tr>
<td>KAUZ</td>
<td>41.6</td>
<td>41.3</td>
<td>41.5</td>
<td>-0.7</td>
</tr>
<tr>
<td>HD 2733</td>
<td>38.1</td>
<td>36.5</td>
<td>37.3</td>
<td>-4.1</td>
</tr>
<tr>
<td>WH 730</td>
<td>44.3</td>
<td>41.1</td>
<td>42.7</td>
<td>-7.2</td>
</tr>
<tr>
<td>Mean</td>
<td>42.4</td>
<td>39.2</td>
<td>40.8</td>
<td></td>
</tr>
</tbody>
</table>

Factors | Genotypes (A) | Treatments (B) | (A X B) |
--------|---------------|----------------|---------|
C.D.    | 4.585         | 2.9            | N/A     |
4.5. Discussion

The average temperature of the Earth is rising owing to climate change. The rise in ambient temperature is inducing changes in the patterns of rainfall, droughts and submergence stress. As a result, growth pattern and thus final yield of crop plants is being negatively affected. Despite the fact that plants have the capacity to evolve and cope up with temperature fluctuations, this capacity might not keep pace with global warming (Grover et al., 2013). Large differences in yields between areas with cooler temperature and warmer temperature or between winter and summer in spite of adequate supply of water shows the significance of high temperature stress in reducing yields (Paulsen, 1994). Heat stress during the post anthesis grain filling stage affects availability and translocation of photosynthates to the developing kernel and starch synthesis and deposition within the kernel, resulting in lower grain weight and altered grain quality (Bhullar and Jenner, 1985). In the present experiment an attempt was made to study the effect of high temperature stress on physiological and biochemical traits, and yield attributes in five contrasting wheat genotypes on the basis of thermotolerance viz. PBW 550, RAJ 3765, KAUZ, HD 2733 and WH 730, with major focus on activity and expression analysis of soluble starch synthase enzyme (SSS). High temperature hastened the decline in photosynthesis and leaf area, decreased shoot and grain weight, and reduced water-use efficiency in wheat (Shah and Paulsen, 2003). Photosynthetic capacity is determined by total leaf area as well as photosynthesis per unit surface area. Simpson (1968) reported that the leaf area was positively and highly correlated with grain yield of many cultivars of wheat. In the present study the leaf area was more reduced during early stages of grain filling in HD 2733 but decline in leaf area was more in later stages of grain filling in RAJ 3765, KAUZ and WH 2733. The lower reduction in leaf area during later stages of grain filling in genotype HD 2733 might caused higher photosynthetic rate and higher stem reserve leading to more mobilization (Blum et al., 1994) leading to more grain filling and ultimately higher grain yield per plant.

Late sowing induced high temperature stress caused greater increase in sugar content in grains of wheat genotypes (Krishna et al., 2011). Grain analysis in our
experiment at three different stages (15, 20 and 25 days after anthesis) of grain filling under high temperature stress also showed the same results. The temperature stress caused greater increases in total sugar in genotypes HD 2733 and PBW 550, while the changes were less in KAUZ and WH 730. Reducing sugar content was high in initial stages of grain filling and there after declined in all the genotypes of wheat studied. The reduction in yield is mainly caused by a reduction in starch accumulation. The reduction in grain weight in response to drought or heat stress during the early periods of grain filling can mainly be attributed to the lower number of endosperm cells (Nicolas et al., 1985), while during the later stages stress results in the impairment of starch synthesis either because of the limited supply of assimilates for the grain (Blum, 1998) or the direct effects on the synthetic processes in the grain (Yang et al., 2004). The process of grain filling, the accumulation of reserve nutrients in the developing and maturing grain, is also sensitive to environmental conditions strongly affecting final yield quantitatively and qualitatively as well (Yang and Zhang, 2006). Starch is a major determinant of yield, accounting for 65-75% of the grain dry weight and up to 80% of the endosperm dry weight (Rahman et al., 2000; Slattery et al., 2000). Diminished rates of starch production in wheat endosperm are also reported to be due to heat inactivation of starch synthase, a key enzyme in the starch biosynthetic pathway (Labuschagne et al., 2009). A greater decline in sugar to starch conversion may have resulted in greater accumulation of sugars in HD 2733. However, starch content increased with growth stages studied in all the genotypes in both control and heat stress treatment. Decline in sugar content during developmental stages may be correlated to more utilization of assimilate translocation for grain sink activity under high temperature stress (Asthir et al., 2012). The total starch content increased significantly from day 15 to day 25 after anthesis under both the control as well as treated plants in all the genotypes studied. Decrease in starch accumulation under high temperature stress is reflected clearly by decline in SSS enzyme activity more prominently in RAJ 3765 and HD 2733 especially during early stages of grain filling (15 and 20 DAA). Comparison of grain weight pattern during high temperature stress with the synthesis of starch in developing grains revealed decrease in starch and increase in total sugars content in the grains of all the wheat genotypes
studied. But the decreases in starch and sugar content under high temperature stress condition were considerably less in tolerant genotypes WH 730 at 20 and 25 DAA.

Grain filling is mainly a process of starch biosynthesis and accumulation. It is generally accepted that four enzymes may play a key role in this process: Sucrose Synthase (SuSase), ADP Glucose Pyrophosphorylase (AGPase), Starch Synthase (SS), and Starch Branching Enzyme (SBE); (Hawker and Jenner, 1993; Ahmadi and Baker, 2001; Hurkman et al., 2003). In the process of starch synthesis, SuSase catalyses the cleavage of Sucrose, the main transported form of assimilates in wheat plants (Fisher and Gifford, 1986), to form UDP-Glucose and Fructose, which is thought to be the first step in the Sucrose-to-starch conversion. AGPase produces ADP-Glucose, the primer of the starch chain (Smith and Denyer, 1992), and is regarded as the rate-limiting enzyme in starch biosynthesis (Preiss, 1988). The decline in starch content for Australian wheat varieties exposed to temperatures greater than 35°C was associated with a decrease in rate of conversion of sucrose to starch (Bhullar and Jenner, 1986). A survey of 11 enzymes of sugar and starch metabolism extracted from developing endosperm revealed that AGPase, glucokinase, SuSase, and SSS were most sensitive to the high temperature treatment. Results indicate that chronic heat stress during grain filling moderately restrains seed storage processes and select enzymes of starch metabolism to similar degrees across multiple maize inbreds (Wilhelm et al., 1999). SSS regulates the synthesis of starch and is sensitive to heat stress (Rijven, 1986; Keeling et al., 1993, 1994). Heat stress decreases the activity of SSS in wheat, reducing grain growth and starch accumulation (Prakash et al., 2003, 2004). Even short periods of episodic temperature over 30°C slows starch accumulation principally due to heat induced denaturation of SSS (Jenner, 1994). Pandey et al. (2012) also confirmed that SSS is the most sensitive enzyme and which can be manipulated further for betterment of wheat crop against heat stress. The results from our studies showed that high temperature stress induced decline in starch accumulation and enzyme activity were higher at 15 DAA in RAJ 3765, KAUZ and HD 2733. It has further emerged that wheat genotype KAUZ, which was having higher yield (grain weight per plant) also have high thermostability (rate of percent change in SSS activity during grain filling). This SS activity can be
correlated with the variability in starch accumulation pattern in wheat grains under high temperature stress condition. Very high decline in enzyme activity was shown by HD 2733 throughout the grain filling period. Kumari et al., (2012) also reported high SSS activity associated with tolerance during drought in wheat. We also studied the expression of SSS family members in wheat under high temperature stress and the transcript levels of SSSI, SSSII and SSSIII at two stages showed good correlation with the activity of SSS, suggesting the regulatory role of SSS in grain starch synthesis and grain growth in wheat genotypes under high temperature stress condition. At 15 DAA under high temperature stress, lesser expression for SSSI and SSSIII was shown by PBW 550 and for SSSII by Raj 3765. However, at 20 DAA same trend for SSSI was shown by PBW 550, but for SSSII and SSSIII isoforms lesser expression was shown by HD 2733. Thus expression analysis results indicate that under high temperature stress SSS of genotypes PBW 550 and HD 2733 is more susceptible than other genotypes used. Quantitative trait locus (QTL) analysis of the japonica and indica rice varieties, which differ in amylopectin structure, suggested that a gene for SSSII (SSSIIa) is responsible for the varietal difference (Umemoto et al., 2002). Temperature may also influence the rate of grain fill, depending on the wheat cultivar. The increase in the rate of grain dry matter accumulation may even compensate for the decrease in its duration (Dupont and Altenbach, 2003). Heat stress reduced both the grain growth duration (GGD) and the grain growth rate (GGR) (Viswanathan and Khanna-Chopra, 2001). In the current experiment, harvested grains showed high temperature induced decrease in GGR in all genotypes studied. Yield attributes were significantly affected by high stress. Dias and Lidon (2000) reported that exposure to high temperature significantly decreased grain weight and hastened physiological maturity, i.e., shortening the grain filling period in bread and durum wheat. Lower seed-set at elevated temperature resulted in fewer filled grains, lower grain weight per panicle, and decreased HI (Prasad et al., 2006). This was also supported by Pandey and Srivastava (2009) where they reported high temperature induced reduction in grain weight in various genotypes of wheat. In our studies a significant decline was observed in grain weight per plant, total biomass and 1000-grain weight and harvest index (HI) in high temperature stress treated plants. The total
biomass decline was lesser for PBW 550 and HD 2733. Higher decline in HI and grain yield per plant was there in RAJ 3765; however, maximum decline in grain yield per plant was in WH 730. HI was markedly reduced by heating and its variation may be associated with changes in stem reserves. Decline was observed in HI under high temperature stress condition in all the genotypes, with maximum decline in thermosensitive genotype (HD 2733). Similar kind of explanation provided that grain yield of maize under high temperature experiment, was better explained by the variation in HI than by the variation in final shoot biomass. Heat effects on grain yield were larger when they occurred around flowering than during grain filling (Juan et al., 2012). Grain yield was negatively related to the thermal time accumulated above the base temperature of 3°C (Ferris et al., 1998, Mian et al., 2007). High temperature above 32°C has been reported reducing grain yield and grain weight (Bluementhal et al., 1995, Gibson and Paulsen, 1999, Wardlaw et al., 2002).

4.6. Conclusion

The results showed that high temperature stress at the time of grain filling affected starch synthesis in the kernels, as reflected by decline in grain starch content and increase in sugar content, and these negative influences of heat stress was more in HD 2733. The results thus showed that KAUZ and WH 730 have the ability to withstand high temperature stress during grain filling phases resulting in higher yield stability than other genotypes studied. Present investigation suggests that high temperature stress induced decline in GGR, grain weight per plant, 1000-grain weight and an overall decline in HI and total biomass in all wheat genotypes. Decline in grain weight and starch synthesis may be due to weak conversion system of sugars into starch manifested by decline in activity of SSS, high temperature sensitive enzyme. Results of lower SSS activity could be correlated to the lesser expressions of SSSI, SSSII and SSSIII under high temperature stress during grain filling period. The differential transcriptional expression of SSS family members in tolerant and susceptible genotypes under high temperature stress provide us with a tool for possible use of these isoforms in increasing grain growth under normal and temperature stress environments.
Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001; Kurek et al., 2007). Heat stress during the post anthesis grain filling stage affects availability and translocation of photosynthates to the developing kernel and starch synthesis and deposition within the kernel, resulting in lower grain weight and altered grain quality (Bhullar and Jenner, 1985). Continuous high-temperature stress for wheat has been defined as when the mean average temperature of the coolest month is greater than 17.5°C (Fischer and Byerlee, 1991), but there are many areas worldwide are exposed to terminal heat stress, since there is rise in temperatures during grain filling period (Rane et al., 2007). In the present investigation an attempt was made to study the effect of high temperature stress on physiological and biochemical traits, and yield attributes in five contrasting wheat genotypes on the basis of thermotolerance viz. PBW 550, RAJ 3765, KAUZ, HD 2733 and WH 730, with major focus on activity and expression analysis of soluble starch synthase enzyme (SSS). The results obtained in the present study, conducted with five wheat genotypes revealed differential response of SSS family members to high temperature stress.

High temperature hastened the decline in photosynthesis and leaf area, decreased shoot and grain weight, and reduced water-use efficiency in wheat (Shah and Paulsen, 2003). Photosynthetic capacity is determined by total leaf area as well as photosynthesis per unit surface area. Simpson (1968) reported that the leaf area was positively and highly correlated with grain yield of many cultivars of wheat. In the present study the leaf area was more reduced during early stages of grain filling in HD 2733 but decline in leaf area was more in later stages of grain filling in RAJ 3765, KAUZ and WH 733. The lower reduction in leaf area during later stages of grain filling in genotype HD 2733 might caused higher photosynthetic rate and higher stem reserve leading to more mobilization (Blum et al., 1994) leading to more grain filling and ultimately higher grain yield per plant.

The temperature stress caused greater increases in total sugar in genotypes HD 2733 and PBW 550, while the changes were less in KAUZ and WH 730. Reducing sugar content was high in initial stages of grain filling and there after
declined in all the genotypes of wheat studied. Significant reduction in total sugars was observed during grain development in both the treatments of wheat. High temperature reduced both content and yield of starch in grains (Zhao et al., 2008). Diminished rates of starch production in wheat endosperm are also reported to be due to heat inactivation of starch synthase, a key enzyme in the starch biosynthetic pathway (Labuschagne et al., 2009). A premature cessation of starch deposition occurred under late sown conditions due to shortening of grain filling period. Decline in sugar content during developmental stages may be correlated to more utilization of assimilate translocation for grain sink activity under high temperature stress (Asthir et al., 2012).

A greater decline in starch synthesis activity must have resulted in greater accumulation of sugars in HD 2733. However, starch content increased with growth stages studied in all the genotypes in both control and heat stress treatment. The total starch content increased significantly from day 15 to day 25 after anthesis under both the control as well as treated plants in all the genotypes across the board. Decrease in starch accumulation under high temperature stress is reflected clearly by decline in SSS enzyme activity more prominently in RAJ 3765 and HD 2733 especially during early stages of grain filling (15 and 20 DAA). Comparison of grain weight pattern during high temperature stress with the synthesis of starch in developing grains revealed decrease in starch and increase in total sugars content in the grains of all the wheat genotypes studied. But the decreases in starch and sugar content under high temperature stress condition were considerably less in tolerant genotypes WH 730 at 20 and 25 DAA.

Grain filling is mainly a process of starch biosynthesis and accumulation. It is generally accepted that four enzymes may play a key role in this process: Sucrose Synthase (SuSase), ADP Glucose Pyrophosphorylase (AGPase), Starch Synthase (SS), and Starch Branching Enzyme (SBE); (Hawker and Jenner, 1993; Ahmadi and Baker, 2001; Hurkman et al., 2003). In the process of starch synthesis, SuSase catalyses the cleavage of Sucrose, the main transported form of assimilates in wheat plants (Fisher and Gifford, 1986), to form UDP-Glucose and Fructose, which is thought to be the first step in the Sucrose-to-starch conversion. AGPase produces ADP-Glucose, the primer of the starch chain (Smith and Denyer, 1992), and is regarded as the rate-limiting enzyme in starch biosynthesis (Preiss, 1988).
A survey of 11 enzymes of sugar and starch metabolism extracted from developing endosperm revealed that AGPase, glucokinase, SuSase, and SSS were most sensitive to the high temperature treatment. Results indicate that chronic heat stress during grain filling moderately restrains seed storage processes and select enzymes of starch metabolism to similar degrees across multiple maize inbreds (Wilhelm et al., 1999). However, Wardlaw and Wrigley (1994) reported that SSS emerged in the workshop as a key enzyme in starch synthesis of cereal grains. Although work is still needed on the function of SSS as a control point in starch synthesis and in the kinetics of its response to temperature, this appears to be the enzyme most strongly associated with the high temperature response of the grain in relation to yield. Pandey et al. (2012) also confirmed that SSS is the most sensitive enzyme and which can be manipulated further for betterment of wheat crop against heat stress. The results from our studies showed that high temperature stress induced decline in starch accumulation and enzyme activity were higher at 15 DAA in RAJ 3765, KAUZ and HD 2733. It has further emerged that wheat genotype KAUZ, which was having higher yield (grain weight per plant) also have high thermostability (rate of percent change in SSS activity during grain filling). This SS activity can be correlated with the variability in starch accumulation pattern in wheat grains under high temperature stress condition. Very high decline in enzyme activity was shown by HD 2733 throughout the grain filling period. Kumari et al., (2012) also reported high SSS activity associated with tolerance during drought in wheat.

We also studied the expression of SSS family members in wheat under hisgh temperature stress and the transcript levels of SSSI, SSSII and SSSIII at two stages showed good correlation with the activity of SSS, suggesting the regulatory role of SSS in grain starch synthesis and grain growth in wheat genotypes under high temperature stress condition. At 15 DAA under high temperature stress, lesser expression for SSSI and SSSIII was shown by PBW 550 and for SSSII by Raj 3765. However, at 20 DAA same trend for SSSI was shown by PBW 550, but SSSII and SSSIII isoforms lesser expression was shown by HD 2733. Thus expression analysis results indicate that under high temperature stress SSS of genotypes PBW 550 and HD 2733 is more susceptible to high temperature than other genotypes used.

There is decrease in transcript levels for the enzyme AGPase and reduction in starch accumulation that accompanies high temperatures during grain filling in
wheat. Zhao et al., (2008) reported that high temperature reduced both content and yield of starch in grains, while it enhanced protein content in grain. There are at least three isoforms of SSS, namely SSSI, SSSII and SSSIII, and the relative activities of these isoforms greatly vary depending on the plant species and tissues (Smith et al., 1997). Quantitative trait locus (QTL) analysis of the japonica and indica rice varieties, which differ in amylopectin structure, suggested that a gene for SSSII (SSSIIa) is responsible for the varietal difference (Umemoto et al., 2002). In maize, a lesion of the gene encoding SSIII (Dull I) causes accumulation of the starch in a form of intermediate-sized highly branched polyglucans (Wang et al., 1993; Gao et al., 1998). In some plant species, including wheat and maize, multiple genes encode each starch synthase isoform, and constitute the starch synthase gene family (Harn et al., 1998; Vrinten and Nakamura, 2000). This implies that the members of the starch synthase gene family are expressed in an organ and development specific manner.

Post-anthesis heat stress in wheat induces several physiological effects which eventually result in smaller grain size due to reduced grain filling period and reduced grain filling rate or the combined effect of both (Hasan and Ahmed, 2005). The effect of heat stress on the components of grain weight was analysed in wheat (varieties differing in grain weight stability). Heat stress reduced both the grain growth duration (GGD) and the grain growth rate (GGR) (Viswanathan and Khanna-Chopra, 2001). Although high temperatures accelerate growth (Fischer, 1980; Kase and Catsky, 1984), they also reduce the phenology, which is not compensated for by the increased growth rate of plant (Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003). In the current experiment, harvested grains showed high temperature induced decrease in GGR in all genotypes studied. Yield attributes were significantly affected by high stress. Dias and Lidon (2009) reported that exposure to high temperature significantly decreased grain weight and hastened physiological maturity, i.e., shortening the grain filling period in bread and durum wheat. Seed-set (spikelet fertility) is an important component of yield that is sensitive to high temperature. In rice also high temperature significantly decreased seed-set across all cultivars, but effects varied among cultivars. Decreased seed-set and cultivar difference at high temperature were mainly due to decreased pollen production and pollen reception (pollen numbers on stigma). Lower seed-set at elevated temperature resulted in fewer filled grains, lower grain weight per panicle, and decreased HI (Prasad et al., 2006).
This was also supported by Pandey and Srivastava (2009) where they reported high temperature induced reduction in grain weight in various genotypes of wheat. In our studies a significant decline was observed in grain weight per plant, total biomass and 1000-grain weight and harvest index (HI) in high temperature stress treated plants. The total biomass decline was lesser for PBW 550 and HD 2733. Higher decline in HI and grain yield per plant was there in RAJ 3765; however, maximum decline in grain yield per plant was in WH 730. HI was markedly reduced by heating and its variation may be associated with changes in stem reserves. Decline was observed in HI under high temperature stress condition in all the genotypes, with maximum decline in thermosensitive genotype (HD 2733). Similar kind of explanation provided that grain yield of maize under high temperature experiment, was better explained by the variation in HI than by the variation in final shoot biomass. The effect of high temperatures on HI and morphological components that contribute to HI was investigated also in cereal model Brachypodium distachyon, a C₃ grass (recognized as a tractable plant, to address critical issues associated with enhancing cereal crop yields in the presence of global climate change). The results demonstrated that temperatures ≥32°C eliminated HI (Harsant et al., 2013).
The present investigation entitled **“Association between the expression of soluble starch synthase family members and grain weight in wheat under high temperature stress”** was conducted to understand and identify isoforms of soluble starch synthase (SSS) family member related to grain weight and thermostability in wheat genotypes during high temperature stress. The differential regulation by different isoforms of SSS family members could further be used by plant breeders and biotechnologists as biochemical/molecular marker for thermostability. In the present study five wheat genotypes differing in terms of high temperature tolerance were selected *viz.* PBW 550, RAJ 3765, KAUZ, HD 2733 and WH 730.

The plants were grown in pot culture, Division of Plant Physiology, IARI New Delhi, from November to May. The plants were subjected to temperature stress by shifting pots to polyhouse (average day/night temperature difference 2.6/1.5°C compared to ambient, during grain filling period). Heat stress treatments provided from anthesis to harvest. Biochemical analysis of grains at 15, 20 and 25 days after anthesis (DAA) was done for estimation of various biochemical and molecular traits such as total sugar, reducing sugar, non-reducing sugar, starch content, extraction estimation of SSS activity, RT-PCR expression analysis of multi-gene members of SSS. Growth parameters like leaf area, grain weight at an interval of 5 days from 15 DAA for grain growth rate (GGR), plant total biomass, grain yield per plant, 1000-grain weight and harvest index (HI) were also studied.

Summary of the results obtained in the present investigation are presented below:

- Leaf area was more reduced during early stages of grain filling in HD 2733 but more decline in leaf area at later stages of grain filling in RAJ 3765, KAUZ and WH 2733.
- The temperature stress caused greater increases in total sugar in genotypes HD 2733 and PBW 550, while the changes were less in
KAUZ and WH 730. A greater decline in starch synthesis activity must have resulted in greater accumulation of sugars in HD 2733.

- Starch content increased with growth stages in both the treatments, irrespective of genotypes. However, under high temperature stress starch accumulation was less as compared to control.

- Decrease in starch accumulation under high temperature stress is reflected clearly by decline in SSS enzyme activity more prominently in RAJ 3765 and HD 2733 especially during early stages of grain filling (15 and 20 DAA).

- High temperature stress induced decline in starch accumulation and SSS enzyme activity were higher at 15 DAA in RAJ 3765, KAUZ and HD 2733.

- Genotype KAUZ, which showed higher yield (grain weight per plant) and high thermostability for SSS (rate of percent change in SSS activity during grain filling), also showed higher expression of \( SSSII \) and \( SSSIII \) at 20 DAA hence could be said as thermotolerant.

- At 15 DAA under high temperature stress, lesser expression for \( SSSI \) and \( SSSIII \) was shown by PBW 550 and for \( SSSII \) by Raj 3765.

- At 20 DAA again lesser expression for \( SSSI \) shown by PBW 550, but for \( SSSII \) and \( SSSIII \) lesser expression was shown by HD 2733.

- Harvested grains showed decrease in GGR under high temperature stress in all the genotypes studied.

- The total biomass decline was lesser for PBW 550 and HD 2733.

- Higher decline in HI and grain yield per plant was in RAJ 3765; however, maximum decline in grain yield per plant was in WH 730.
Association between the expression of soluble starch synthase family members and grain weight in wheat under high temperature stress

Increases in temperature due to global warming can cause heat stress: a severe threat to wheat production, particularly when it occurs during reproductive and grain-filling phases. The present study was conducted with five different wheat genotypes viz. PBW 550, RAJ 3765, KAUZ, HD 2733 and WH730, in terms of temperature tolerance. Sowing done on 26th November 2012 and pots from each genotype were shifted to polyhouse around 13th February, 2013 (nearly a week prior to anthesis for each genotype) for providing high temperature stress (average day/night temperature of 32.4°C/15.5°C; difference of 2.6°C/1.5°C compared to ambient) at the time of grain filling. The observations were taken at 15, 20 and 25 days after anthesis (DAA). This high temperature stress led to the reduction in growth cycle and yield related attributes in all the genotypes. High temperature stress decreased leaf area subsequently at different growth stages, and biomass and grain yield at harvest in all the genotypes and also found that GGR and starch were significantly reduced but total sugar increased by high temperature stress during grain filling, irrespective of genotypes. However, under temperature stress KUAZ and WH 730 (relatively thermotolerant) showed lesser decrease in level of starch and a less increase in total sugar content, in comparison with other genotypes. The reduction in activity of soluble starch synthase (SSS) in KAUZ and WH 730 was lesser at 20 DAA when compared to 15 and 25 DAA suggested higher expression of some isoform during that period of growth in these genotypes. A comparatively higher reduction was observed in susceptible genotype HD 2733 at same stage of grain filling (20 DAA). Expression analysis of SSS family members at 20 DAA, revealed that in tolerant genotypes (KAUZ and WH 730) expression of isoforms SSSII and SSSIII were at higher levels as compared to susceptible genotype (HD 2733). From the study, it can be concluded that the efficient up-regulation of different isoform of SSS at different stages of grain filling played a major role in imparting high temperature stress tolerance in tolerant wheat genotypes.
उच्च तापमान प्रतिबल के अंतर्गत गेहूँ में घुलनशील स्टार्च सिंथेज कुल के सदस्यों की अभिविक्तियों एवं दाना भार के बीच साझेदारी

सार

भूमण्डलीय तापमान के कारण तापमान में बढ़ोतरी के कारण ताप प्रतिबल उत्पन्न हो सकता है जो गेहूँ के उत्पादन में विशेष रूप से जब यह पुनरुत्पादन एवं दाना भरने वाली अवस्था में होता है, एक गंभीर चुनौती है। ताप सहनशीलता के सन्दर्भ में यह अध्ययन गेहूँ के पांच मिला-मिला जीनप्ररूपों यथा, पी.बी.डब्ल्यू-550, आर.ए.जी-3765, के.ए.यू.जी, एच.डी. -2733 एवं डब्ल्यूएच-730 के साथ किया गया। इनमें 26 नवम्बर, 2012 को की गई तथा दाना भरने के समय उच्च तापमान प्रतिबल (32.4 डिग्री सेल्सियस/15.5 डिग्री सेल्सियस का औसत दिन/रात तापमान; परियोजना तापमान से क्रमशः 26 डिग्री सेल्सियस/1.5 डिग्री सेल्सियस अधिक) उपलब्ध कराने के लिए 13 फरवरी, 2013 को प्रारंभ जीनप्ररूप के गमनों को पॉलीहाइड्रो में स्थानान्तरित कर दिया गया। परागोदन के 15, 20 एवं 25 दिन बाद (डी.ए.) इसका निरोपण किया गया। उच्च तापमान प्रतिबल के परिणामवर्गण सभी जीनप्ररूपों के वृद्धि चक्र एवं उपज संबंधी गुणों में कमी देखी गई। सभी जीनप्ररूपों में उच्च तापमान प्रतिबल के कारण विभिन्न वृद्धि अवस्थाओं में पर्यंत क्षेत्रफल, जैविक पदार्थ तथा कादम के पश्चात दाना उपज में कमी पायी गई। दाना भरने के दौरान विभिन्न समयांतरालों पर दाना वृद्धि दर (जी.जी.आर.), दानों के कुल शार्करा एवं स्टार्च अंश भी देखे गए तथा यह पाया गया कि सभी जीनप्ररूपों में दाना भरते समय उच्च तापमान प्रतिबल के कारण जी.जी.आर. एवं स्टार्च अंश में महत्वपूर्ण रूप से कमी आई किन्तु कुल शार्करा अंश में बढ़ोतरी हुई। तापमान प्रतिबल के अंतर्गत, अन्य जीनप्ररूपों की तुलना में के.यू.ए.जी. एवं डब्ल्यूएच-730 (अपेक्षाकृत ताप सहनशील) ने स्टार्च स्तर में कम कमी वर्षीय तथा कुल शार्करा अंश में बढ़ोतरी भी कम हुई।

15 एवं 25 डी.ए. की तुलना में 20 डी.ए. पर के.यू.ए.जी. एवं डब्ल्यूएच-730 में घुलनशील स्टार्च सिंथेज (एस.एस.एस.) की सक्रियता में कमी कम पायी गई जो इन जीनप्ररूपों में वृद्धि की अवधि के दौरान कुछ आइसोफॉर्म की उच्चतर अभिविक्ति का सुझाव देती है। दाना भरने की उसी अवस्था (20 डी.ए.) पर सुग्राही जीनप्ररूप एच.डी-2733 में तुलनात्मक रूप से उच्चतर अभिविक्ति देखा गया। 20 डी.ए. पर एस.एस.एस. कुल के सदस्यों के अभिविक्ति विकल्पना ने दर्शाया कि सहनशील जीनप्ररूपों (के.ए.यू.जी. एवं डब्ल्यूएच-730) में सुग्राही जीनप्ररूप (एच.डी-2733) की तुलना में आइसोफॉर्म एस.एस.एस. II एवं एस.एस.एस. III
उच्चतर स्तरों पर थे। इस अध्ययन से यह निष्कर्ष निकाला जा सकता है कि दाना भरने की विभिन्न अवस्थाओं पर एस.एस.एस. के भिन्न-भिन्न आइसोफोर्म का सक्षम अप-रेग्यूलेशन, गेहूँ के सहनपील जीनप्रशस्तियों को उच्च तापमान प्रतिबल हेतु सहनपीलता प्रदान करने में एक प्रमुख भूमिका निभाता है।


