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Terminal Heat Stress Induced Alterations in Starch Synthase Gene Expression in Wheat Genotypes Differing in Thermo-Tolerance

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Abstract—*A field experiment was conducted with three wheat varieties Raj 4014 (heat sensitive), K 7903 and WH 730 (heat toletrant) for characterization of soluble starch synthase (SSSI) under terminal heat stress. Heat stress enhanced the expression of SSSI at early and mid-grain-filling stages such that the peaks of enzyme expression were attained earlier as compared to those under normal conditions. In addition, expression analysis demonstrated that altered SSS transcript level was genotypic dependent.*

Keywords*: wheat, terminal heat stress, starch synthase, thermo-tolerance.*

1. INTRODUCTION

Wheat is a staple food for millions across the world. At present, global wheat production is 716 million tons and needs to be raised to 880 million tons to meet the demand of the projected global population of 9 billion (FAO, 2006: A Report on World Agriculture: towards 2030/2050). While grown in a wide range of agro ecologies and resources continue to be the same or tend to decline, biotic and abiotic factors can continue to be the major constraints in their present form or in the amplified version under changing climate. Among abiotic stresses, heat stress is a primary constraint to global wheat production, particularly in the tropical and subtropical regions of Southeast Asia [8]. All the growth stages of wheat are sensitive to heat stress however; grain development is the most sensitive one. Terminal heat stress affects both grain setting and grain filling [13]. It has been estimated that every 1°C rise in temperature above the optimum reduces the yield

per spike by 3-4% [11]. Grain weight was decreased by 85% under continued heat stress during grain filling [9]. Under heat stress, wheat crop completes its life cycle much quicker than under normal temperature conditions [1]. Under supra optimum temperature, the duration of starch biosynthesis and deposition in grain is reduced. This reduction can almost always be ascribed to decreased starch biosynthetic enzyme activity [14]. Among enzymes of the starch biosynthesis, soluble starch synthase (SSS) is the most sensitive to high temperature [2, 14]. SSS in wheat lost up to 97% activity at 40^0C and becomes non-functional [3]. Further, gene expression analysis in wheat revealed that high temperature highly decreased the transcript number for the SSS than for the other enzymes involved in starch biosynthesis [2]. However, [3] reported that the loss of SSS activity up to 30^0 C is not large enough to reduce starch deposition but could alter its composition. Hence, an understanding of the molecular basis of heat tolerance will assist in the improvement of heat tolerance in wheat breeding programs. Therefore, it would be pertinent to elucidate the molecular mechanism of terminal heat tolerance in bread-wheat. So, the present investigation was planned to study the expression of SSS gene under heat stress condition.

2. MATERIALS AND METHODS

The experiment was conducted at Indian Institute of Wheat and Barley Research, Karnal in the Indo-Gangetic plain in north western India, with mildly alkaline sandy loam (Typic ustochrept) soil over a period of two years (2012-13 and 2013-14). Experiments were laid out in a randomized complete block design with three replications. To expose the plants to different levels of temperatures at the time of grain filling, the crop was sown under optimal irrigated conditions during the second week of November as control and under very late sown (LS) irrigated conditions during the second week of January. The average ambient temperatures during the grain growth phase between anthesis to physiological maturity ranged from 25.1^0C to 25.4^0C when sown in November and 32.0^0C to 32.6^0C when sown in January. Three spring wheat genotypes viz. K 7903, RAJ 4014 and WH 730, with a wide range of genetic background were chosen. K 7903 has been shown to have superior heat tolerance based on its ability to escape stress [8]. RAJ 4014 was recommended for cultivation under timely sown (LS) conditions of North Eastern Plain Zone and does not perform well under LS (terminal heat stress) conditions [6]. WH 730 is recommended for planting under LS conditions of the North Eastern Plain Zone. This genotype is characterized by excellent adaptability to heat stress due to stay green character [6]. Sowing was done with the IIWBR Dibbler to ensure precision in planting (Sharma et al. 2016). Dibbling tool was used to place seeds at one locus without any overlap at 6.5 cm depth within a small experimental unit of 4 rows of 50 cm length with 20 cm space between the rows and 10 cm between the plants within the rows. Seeds were hand-planted in uniformly created cavities at the rate of single seed/cavity at the rate of 24 seeds per plot. Inorganic fertilizers were applied at recommended rates (150 : 60 : 40 kg N: P_2O_5: K_2O/ha).

Weeds were removed manually and all other agronomic practises were carried as per recommendations.

2.1 Seed Sampling

Spikes that flowered on the same day were tagged for each genotype under both timely and late sown conditions. Developing Spikes were sampled in the morning at five day intervals from anthesis to 35 DPA. All grains from each spikelet were removed and frozen in liquid nitrogen for few minutes and then stored at -80^{0}C for expression analysis.

2.2 Isolation of Total RNA and cDNA Synthesis

High quality (free of starch and proteins) RNA in sufficient quantity is a prerequisite for Real-Time PCR analysis. Total RNA from developing wheat grains were isolated as per the method described by [5]. Amount of total RNA in each sample was determined by nanodrop and then concentration of each sample were made identical by dilution with nuclease free water. cDNA was synthesised from total RNA using the iScript^{TM} cDNA Synthesis Kit (BIO-RAD Cat# 170-8890). cDNA synthesis was carried out in the BIO-RAD thermocycler (C1000^{TM} Thermal Cycler).

2.3 Primer designing and PCR amplification

The DNA sequences of SSSI gene were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/). The sequences were multiple aligned by ClustalW software (http://www.ebi.ac.uk/Tools/msa /clustalw2/) to find the conserved sequence and primer was designed from this sequence using Primer Express$^{(R)}$ (Fig.1). Primers were designed to amplify a product of approximately 60 bp size and Tm near to 60°C.

Primer concentration of 100 ng (F): 250 ng (R) resulted in best CT value for *SSSI*. Melt curve didn't indicate the formation of primer dimer as no peak was observed in the control reaction performed without cDNA. Thus, above mentioned primer concentration combination was used for RT-PCR analysis. Actin was used as a loading control. Reaction was performed in a 20 µl volume containing 4 µl of cDNA, 10 µl of 2X Syber green mix PCR buffer, 0.2 µM of each primer. The thermocycling program consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of 20 sec at 95°C, 60 sec at 60°C and a final cycle of 2 min at 60°C in BIO-RAD thermocycler (C1000^{TM} Thermal Cycler). The relative expression level of all the transcripts were calculated using the comparative $2^{-\Delta\Delta Ct.}$ $\Delta\Delta$ C_t was calculated as follow: ΔC_t (treated) = C_t (gene-treated) – C_t (actin-treated); ΔC_t (control) = C_t (gene-control) – C_t (actin-control) and $\Delta\Delta$ C_t = Δ C_t (treated) – C_t (control).

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3. RESULTS AND DISCUSSION

Bread wheat (*Triticum aestivum* L.) is exposed to seasonal fluctuations in temperature, which have potential impacts on yield. A significant yield reduction was observed at an increase of 1.5°C in temperature above optimum [12]. It was reported that an increase of the seasonal average temperature by 1°C decreased the grain yield of cereals by 4.1% to 10.0% [10]. In the present investigation, an attempt has been made to investigating molecular mechanism of heat tolerance. qPCR was done using primers specific for the wheat *SSSI* to examine the accumulation profiles of *SSS1* transcript in developing grains of three wheat genotypes under late sown condition relative to that under timely sown conditions (Fig. 2). The expression of *SSSI* was temporally regulated. A significant change in the starch biosynthetic gene expression was observed in all the cultivars under late sown condition. It was observed that *SSSI* was upregulated in all the three wheat genotypes till 15 DPA having a maximum accumulation at 10 DPA in response to terminal heat stress. At 20 DPA, transcript level in RAJ 4014 and WH 730 remained high while K 7903 showed negative regulation in response to terminal heat stress. However, at 25 and 30 DPA, the expression of *SSI* transcript was highly down regulated in all the three genotypes with K 7903 showing a maximum reduction. This explained the heat escaping behaviour of K7903 and stay green character of WH730. Heat stress enhanced the expression of *SSSI* at early and mid-grain-filling stages such that the peaks of enzyme expression were attained earlier as compared to those under normal conditions. This altered the endosperm development because of changes in the pattern of assimilate translocation, grain-filling duration and rate. Wheat and barley proteome analysis under heat stress also showed the similar pattern of expression of heat stress related proteins [4]. These heat stress responsive changes could adjust the tolerance mechanism of wheat under heat stress at different stages of grain filling. The information revealed in this study could be exploited in molecular breeding for improvement of thermotolerance in bread-wheat.

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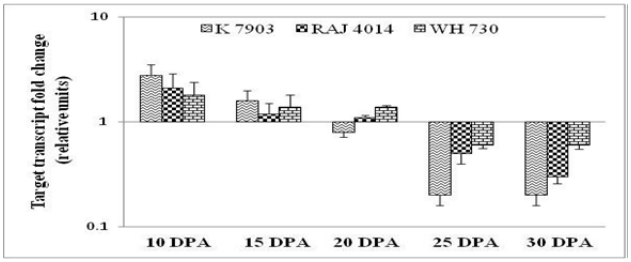

Figure 1. Multiple alignment of *SSSI* sequences with ClustalW. Primers are denoted by underlined sequences.

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gi|9369335|emb|AJ494544.3|
TATTGTTAATGCCATCGAGATTTGAACCTTGCGGTCTTAATCAGCTATAT 3767

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gi|9369335|emb|AJ494544.3|
GCTATGCAATATGGTACAGTTCCTGTAGTTCATGGAACTGGGGGCCTCCG 3837

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gi|9369333|emb|AJ494543.3|
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gi|9369335|emb|AJ494544.3|
AGACACAGTCGAGACCTTCAACCCTTTTGGTGCAAAAGGAGAGGAGGGTA 3867

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gi|9369335|emb|AJ494544.3|
CGAACCGCGATGTCGACATTCAGGGAGCACAAGCCGTCCTGGGAGGGGCT 3967

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TAGACGGGGACTGGGGAGGTCCAAGTGCGAGTCTCCTTGAGCTCTGAAGA 4337

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Figure 2. The relative expression profile of *SSSI* under terminal heat stress condition in wheat. Vertical bars indicate standard error of the mean.