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Transcript Expression Profiling of SnRK1 (Sucrose Non-Fermenting-1-Related Protein Kinase 1) under Terminal Heat Stress in Wheat (*Triticum aestivum* L.)

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ABSTRACT

With ensuing global climate change high temperatures are becoming an important limiting factor of yield and quality and crops will have to adjust to even higher temperatures in the near future. The grain-filling stage of wheat crop is very sensitive to high temperatures. SnRK1 is a key metabolic regulator which plays an important role in plant carbon, nitrogen metabolism and development. This serine/threonine kinases family of enzyme play a central regulatory metabolism by responding to cellular energy change through relative AMP and ATP concentrations. The key role that SnRK1 play at the interface between metabolic and stress signaling makes them potential candidates for manipulation to improve crop performance in extreme environments. In the present study a transcript expression profiling was carried out in cultivar of wheat HD2967. Two vegetative stages and five reproductive-grain development stages were exposed to high temperature stress at 37ºC and individual, independent earheads were utilized for transcript profiling of wheat SnRK1 gene. Contrasting expression was observed with lower expression in early vegetative stages and higher expression for generative stages for HD2967. As a whole, these transcript expression findings show that SnRK1s are involved in diverse physiological processes throughout the life cycle of wheat plants, from the time of seedling establishment to high temperature induction to senescence.

Keywords: *SnRK1, Terminal Heat Stress, Metabolic Sensor, Grain Filling Stage, Reproductive stage.*

INTRODUCTION

Wheat is widely adapted crop and cultivated throughout the world's temperate regions. Wheat prefers relatively cool temperatures and is sown throughout the world in late autumn or early winter and harvested before early summer.

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Thus the temperature in many wheat growing regions rises towards the end[1], during grain filling or reproductive development called end of season or terminal heat stress. During grain-filling stage, high-temperature fluctuations can decrease pollen fertility, grain plumpness, starch content, and protein accumulation, impacting both yield and quality[2]. Thus heat stress and specifically terminal heat stress is one of the top most research priorities in wheat crop today.

The sucrose non fermenting -1 (SnF1)-like kinases in eukaryotes are evolutionary conserved classical proteins which adapt metabolism to environmental conditions such as nutrition, energy, and stress[3]. The protein kinases SNF1(in yeast)/AMPK(in mammals)/SnRK1 (plants) are a subfamily of serine/threonine kinases function as metabolite sensors to constantly adapt metabolism to the supply and demand for energy and plays an important role in plant carbon, nitrogen metabolism and development[4] .In the yeast the SNF1 complex is a central component of the regulatory response to glucose starvation. The mammalian homologue of SNF1, AMP activated protein kinase (AMPK), plays a central role in the regulation of energy homeostasis at the cellular as well as the whole-body levels[3].The plant family is subdivided into three sub-families: SnRK1, SnRK2, and SnRK3[4]. All the sucrose non fermenting protein kinases namely mammalian, yeast, and plant AMPK, SNF1, and SnRK1, respectively exists as heterotrimeric complexes with one α -catalytic and two β- and γ regulatory-subunits[5]. The AMP/ATP ratio conformationaly regulates mammalian AMPK whereas plant SnRK1 is regulated by sugar phosphates[6]. Plants link metabolic and stress signaling through SnRKs in a unique way that does not occur in other organisms. Thus the key role that SnRK1 play at the interface between metabolic and stress signaling make them potential candidates for manipulation to improve crop performance in extreme environments.

In addition to the prospective in developing crops with greater tolerance to stress; SnRK1 also control starch accumulation in storage organs, another potential switch to manipulate carbohydrate metabolism to increase the energy/nutritional value of crops. As suggested by Coello *et al.*[7], increasing flow of carbon into starch and other storage products could enhance sink strength; moderate the build-up of sucrose thereby decreasing feedback inhibition of photosynthesis, maximizing carbon fixation and enhancing yield. Thus modification of the metabolic and stress signaling pathways mediated by the SnRK1 protein kinase in plants has the potential to contribute to improving crop production and productivity. The variety HD2967 released by IARI, New Delhi is grown majorly in North Western and North Eastern Plain Zone for its rust resistant character.

The optimum temperature for wheat anthesis and grain filling ranges from 12 to 25^0C and exposure to temperatures above this can significantly reduce grain yield [8, 9, 10, 11, 12]. Heat stress leads to increased floret abortion during anthesis, pollen sterility, tissue dehydration and lower CO_2 assimilation and increased photorespiration[13,14]. Although high temperatures can accelerate growth, they also reduce the phenology ,which is not compensated for by the increased growth rate[15,16]. Therefore, when temperatures are elevated between anthesis to grain maturity, grain yield is reduced due to the reduction in time to capture resources.

MATERIAL AND METHODS

Seeds of cultivar HD2967 were taken from the Division of Genetics and Division of Agronomy of IARI, New Delhi respectively. Seeds were grown in different 14 independent pots in glass house of phytotron facilities of IARI, New Delhi and at a particular time point shifted to temperature controlled growth chamber .Plants of particular stage exposed to heat stress for 2 hrs at 37^0C. Immediately after the treatment tissue samples were collected from seven stages that are whole seedling stage, flag leaf stage, booting stage, post anthesis,10 days after post anthesis, 20 days

after post anthesis,30 days after post anthesis. All the samples used were from the developing spikelets or earheads from independent pots grown at regular growth conditions and high temperature exposed at 37^0C for2 hrs.

Total RNA was isolated from Different seven stages of cultivar under control and heat treated condition by Trizol Reagent. Quantification and quality checking of RNA was done by spectrophotometric method. cDNA synthesis were made from total RNA by reverse transcriptase. Amplification of target cDNA by PCR was done by using following primer sequences. After performing PCR, amplicons were analyzed on 1.2% TBE agarose gel and gel photograph was taken using syngene gel documentation system.

List of primer sequences used in the study

S.No.	Target amplicon	Primer name	Primer sequence
1.	Full SnRK1 gene	SnRK1FF	5'ATGGATGGGA ACACTAGAGG AGGA3'
		SnRK1FR	5' CTACAGAACCCTAAGGTTGGTAAG 3'
2.	SnRK1 expression	SnRK1exp F	5' TTATTGAAACTCCGTCGGACATA 3'
		SnRK1exp R	5'CTTCATCCTCTTGTAACCTACCC 3'
3.	18S rRNA gene	Ta18S rRNA F	5'TTTGACTCAACACGGGGAAA 3'
		Ta18S rRNA R	5' CAGACAAATCGCTCCACCAA 3'

In order to investigate high temperature induced expression pattern of SnRK1 gene in vegetative and generative stages various tissue samples were harvested under controlled and heat stress condition and transcript expression profiling was carried out by semi quantitative RTPCR.

RESULT AND DISCUSSION

Primer designing and RT-PCR analysis

The various database searches has revealed that no wheat full length SnRK1 gene sequence is available in public databases except for a partial fragment of 527 bp mRNA. This sequence was downloaded along with whole coding sequence of SnRK1 gene from barley, *Braychopodium*, *Arabidopsis*, rice and maize were also downloaded from NCBI (www.ncbi.gov/nucleotide) For transcript expression profiling primer sets were designed from partial sequence of available wheat mRNA and for isolation of full length coding region primers were designed from related species and other cereals. Reverse transcription was carried out using 1ug of stressed and control RNA using oligodT primers and PCR analysis was carried out using equal amount of cDNA (2ul of RT mix). Equal amount of RTPCR products were loaded in each well and subsequently checked on 1.5 % agarose gel. RTPCR was carried out with wheat 18SrRNA gene specific primers as constitutive or housekeeping gene control for determining equal loading and quality control.

Fig.3, shows RTPCR analysis in wheat Cultivar HD2967 using SnRK1 gene specific primers and Fig.4 shows semi quantitative estimation of change in fold expression. Table 1 shows increase and decrease in transcript modulation in treated samples relative to untreated controls. There was less expression of SnRK1 transcripts in whole seedling, flag leaf and booting stage and it showed a rise from post anthesis and continued till thirty days post anthesis. Whole seedlings control showed a 15.92% more expression in than treated samples. There was a decrease in expression in flag leaf and booting stage treated samples when compared to their heat stressed counterparts by 24.92% and 19.10% respectively. Starting post anthesis treated stages it showed increased expression at 47.20%, 1.20%and 50.20% respectively. As the grains moved towards maturity at thirty days post anthesis HD2967 samples showed a reduction in expression at 19.51%.

A continued high induction of SnRK1 was observed post anthesis over the grain development stages in both control and treated samples of HD2967 with a slight dip twenty

days post anthesis.As a whole, these transcript expression findings show that SnRK1s are involved in diverse physiological processes throughout the life cycle of wheat plants, from the time of seedling establishment to high temperature induction to senescence.

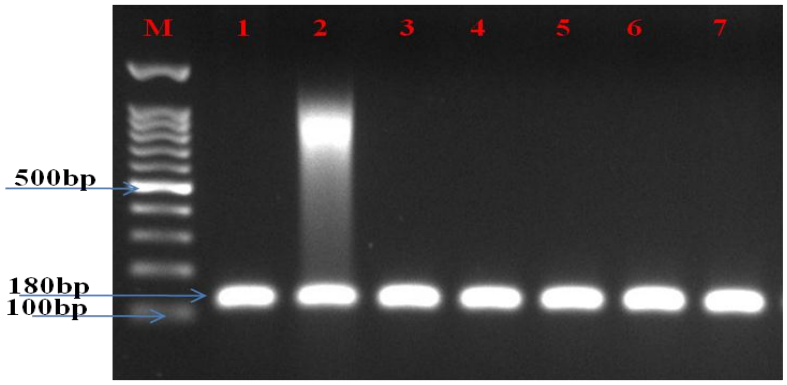

Fig. 1: RT-PCR analysis of cultivar HD2967 grown under normal condition using 18S rRNA gene at all different developmental stages which show equal loading and expression of 18S rRNA in all the samples as shown on 1.2% agarose gel (Lane M : 100 bp ladder ,Lane1:Seedling stage, Lane2: Flag leaf stage, Lane3:Booting stage, Lane4: Post anthesis(0 days), Lane5: 10 days after post anthesis, Lane6: 20 days after post anthesis, Lane7: 30 days after Post anthesis).

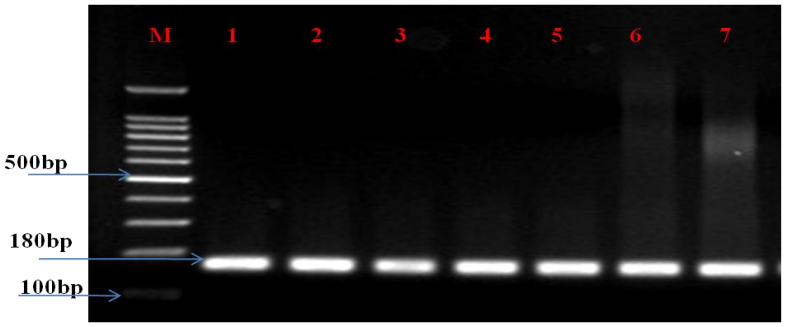

Fig. 2: RT-PCR analysis of cultivar HD2967 grown under heat stress condition using 18S rRNA gene at all different developmental stages which show equal loading and expression of 18S rRNA in all the as samples shown on 1.2% agarose gel (Lane **M : 100 bp ladder ,**Lane**1**:Seedling stage, Lane**2**: Flag leaf stage, Lane**3**:Booting stage, Lane**4**: Post anthesis(0 days), Lane**5**: 10 days after post anthesis, Lane**6**: 20 days after post anthesis, Lane**7**: 30 days after Post anthesis)

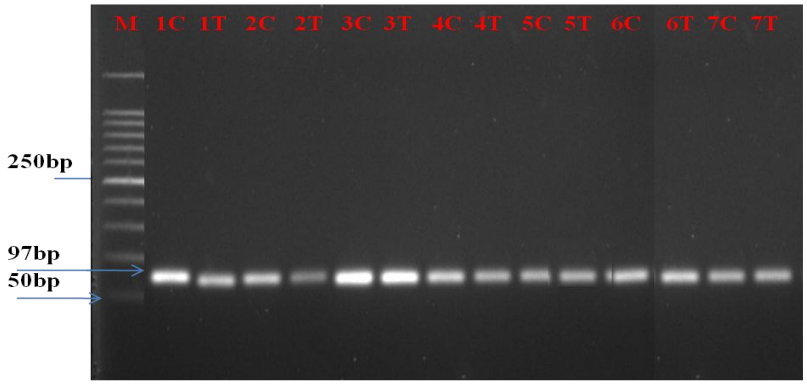

Fig. 3: RT-PCR analysis with SnRK1 gene specific primers in different generative stages of HD2967 Cultivar exposed to high temperature stress at 37^0C for 2hrs along with the control shown on 1.5% agarose gel (Lane **M : 50 bp ladder, C**-control sample ,**T**-heat treated sample ,Lane**1** : Seedling stage, Lane**2**: Flag leaf stage, Lane**3**:Booting stage, Lane**4**: Post anthesis(0 days), Lane**5**: 10 days after post anthesis, Lane**6**: 20 days after post anthesis, Lane**7**: 30 days after Post anthesis)

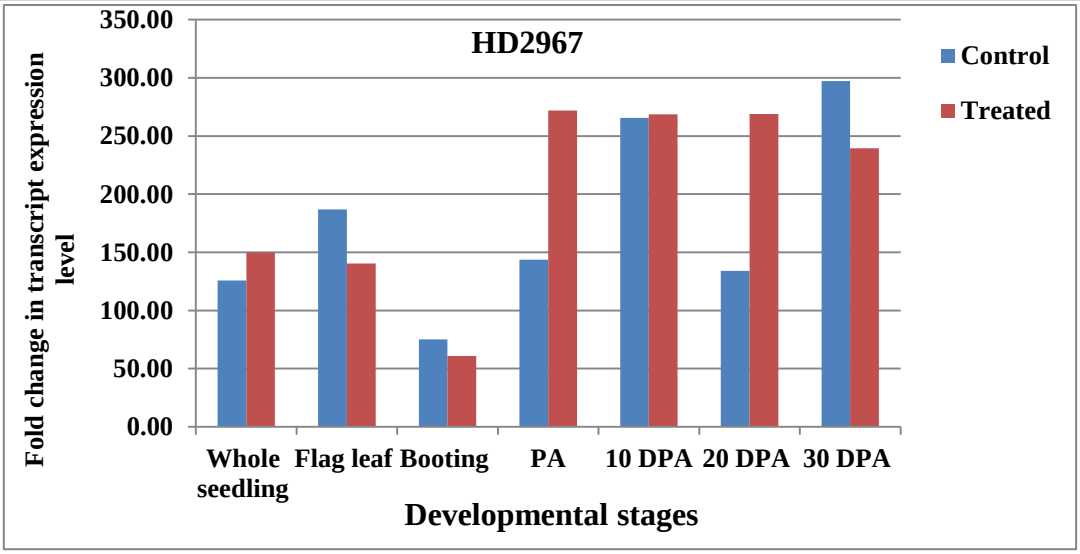

Fig. 4: Semi quantitative transcript expression profiling of HD2967 Cultivar with SnRK1 in different developmental stages under both control and heat treated samples (PA-Post anthesis, DPA-Days after Post anthesis).

Table 1 : Transcript expression modulation of *Ta*SnRK1 in cultivar HD2967 showing the absolute densiometric values measured by Gene tool Software (Syngene,UK).The percentage increase or decrease in treated samples over control samples is indicated as (+)increase and(-) as decrease.

S.No.	Stages	Absolute value		Fold change* (%) in (T) over (C)
		Control(C)	Treated(T)	
1.	Whole seedling	125.84±14.60	149.66±4.90	-15.92
2.	Flag leaf	186.99±14.99	140.40±7.46	24.92
3.	Booting	75.21±2.02	60.84±7.78	19.10
4.	Post anthesis	143.58±8.71	271.91±22.56	-47.20
5.	10 days after PA	264.45±2.11	268.68±0.56	-1.20
6.	20 days after PA	133.92±1.78	268.92±16.27	-50.20
7.	30 days after PA	297.33±5.82	239.31±11.50	19.51

*Data is taken as mean of three replicates and ”minus(-)” indicates reduction in fold change in percentage.

CONCLUSION

An active functional role for SnRK1 in pre-grain development and throughout grain development stages under regular growth conditions and under high temperature exposure has been indicated through extensive transcript profiling. Different expression of SnRK1 in both the stages can be attributed to their critical regulatory role in pre and post grain filling as well as in stage transitions in wheat plants. Identifying the interacting partners and targets of SnRK1 during these physiological stages of wheat crop may explain the differential response in the different wheat cultivars . Further functional analysis *in vivo* will clarify their role in sugar and hormonal signaling in grain development under terminal heat stress.

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