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SHORT RESEARCH ARTICLE

Isolation and characterization of novel gene *TaSSRP* differentially expressed in wheat (*Triticum aestivum* L.) genotypes under heat stress

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Abstract

Analysis of heat stress-responsive transcriptome data in wheat genotype Raj 3765 revealed that expression of salt stress root protein (*SSRP*) RS1 was observed to increase by 8.29 folds in the flag leaf at the grain filling stage. Further validation of the gene under heat stress condition (42°C for 6 hours) by qRT-PCR revealed an increase of 8.42 folds. *In-silico* studies revealed that the gene is localized mainly in the cytoplasm, and did not contain transmembrane helix. The presence of 15 phosphorylation sites as indicated by *in-silico* analysis could play significant roles in the heat stress tolerance.

Keywords: Heat stress tolerance, *In-silico, TaSSRP*, wheat

Wheat (*Triticum aestivum* L.) is one of the world's staple crops and is ranked as the third-largest food crop worldwide in terms of production. Temperature above 35°C adversely affects most wheat genotypes, especially during the grain filling, which shortens grain filling duration and reduces grain yield and quality (Zhao et al. 2017). Consequently, heat stress has become a great hindrance to successful crop production globally (Gourdji et al. 2013). In the present study, a novel gene *TaSSRP* (a salt stress root protein RS1 with contig ID 6781) was identified in transcriptome sequencing data generated in our laboratory (SRA number: SRR16347581and SRR16347579) was characterized for its role in heat stress tolerance in wheat.

A panel of fifteen released wheat genotypes contrasting for heat stress tolerance used in the study were grown and maintained under greenhouse conditions at the National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. Leaf samples were collected from three biological replicates of each genotype at the post-anthesis stage (Feekes scale: 10.53) after giving heat stress (HS) at 42°C for six continuous hours in an incubator chamber. The plants grown without HS treatment (24 ± 2°C) were used as control. Total RNA was isolated using Spectrum™ Plant Total RNA kit (Sigma Aldrich, USA) as per manufacturer's protocol, and the quality and concentration of the total RNA were checked by electrophoresis and NanoDrop (Thermofischer Scientific, USA), respectively. DNase treatment (Sigma-Aldrich, USA) was given, and cDNA was synthesized using Superscript III first-strand cDNA synthesis system (Invitrogen, USA). Primers for qRT-PCR analysis of gene *TaSSRP*, found to be up-regulated in heat stress-responsive wheat transcriptome data were designed using the available EST sequence and Primer3 software (Forward: ATGACGAGCGTATGGAAGACC; Reverse: TCACTTCTTCTCTGGCTCCTC). qRT-PCR was carried out using the gene specific primers. β-actin gene (accession no. AB181991.1) was used as internal control. Each reaction was performed in triplicates and relative fold change values

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were calculated by $2^{-\Delta\Delta Ct}$ method. The full-length CDS of gene TaSSRP was isolated from *Triticum aestivum* cv. Raj 3765, cloned, transformed into *E. Coli DH5a* cells by heat shock method (Froger and Hall 2007) and sequenced by Sanger sequencing. *In-silico* studies of TaSSRP were conducted to determine physico-chemical properties, subcellular location, post translational modifications, transmembrane helices, secondary and 3D structures, and Ramachandran plot.

The results showed that, in exception of *Dharwad* Dry, the expression of the gene *TaSSRP* using qRT-PCR was up-regulated in response to heat stress in all the genotypes at 42 °C for 6 h (Fig. 1). The highest expression of 8.42 folds was recorded in wheat cv. Raj 3765, which is a well-known heat tolerant wheat variety in India. This may indicate a protective mechanism against protein denaturation due to heat stress.

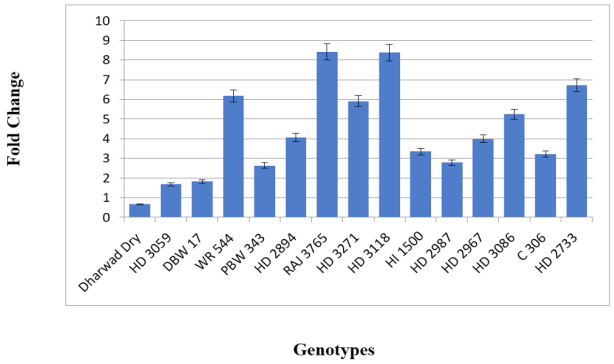

Fig. 1. Gene expression analysis by qRT-PCR of gene *TaSSRP* in different wheat genotypes under heat stress conditions

TaSSRP (CDS: 636 bp; accession number: MT341468) was determined to be mostly localized in the cytoplasm (Supplementary Table S1). There was no transmembrane helix in *TaSSRP* (Supplementary Fig. 1), which could be one of the reasons for it not being targeted to any particular organelle. Vishwakarma et al. (2018) also reported absence of transmembrane helix in *TaHsp90* sequence found to be involved in heat stress tolerance.

The *TaSSRP* protein showed a total of 15 phosphorylations; 8 of serine, and 7 of threonine (Supplementary Fig. 2A). The 3 potent N-linked glycosylated sites (Supplementary Fig. 2B) and six O-linked glycosylation sites (Supplementary Fig. 2C) were predicted. The phosphorylated and glycosylated sites identified in *TaSSRP* are believed to be functional in heat stress tolerance in wheat genotype Raj 3765. According to Goswami et al. (2016), post-translational modifications (PTMs) may be used in manipulating the expression of genes/proteins associated with thermotolerance.

The secondary structure prediction indicated that *TaSSRP* had 125 (59.24%) α-helices, 7 (3.32%) extended strand regions, and 79 (37.44%) random coil regions (Supplementary Table S2). Secondary structure generated by PSIPRED tool was about 90% disordered (Fig. 2A). A total of 16 residues (7.5% of the sequence) have been

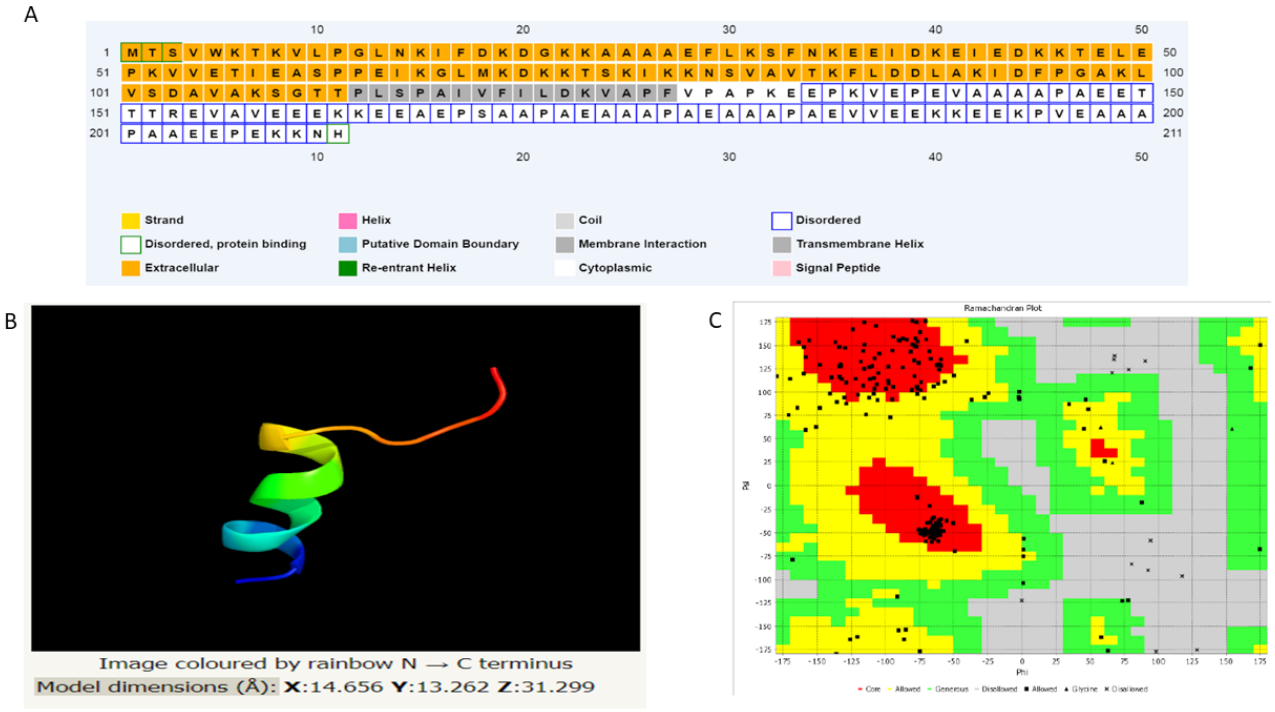

Fig. 2. Protein structure prediction: (A) Secondary structure map of *TaSSRP* showing helices, sheets and disorderness; (B) 3D Modelling of *TaSSRP* And (C) Ramachandran plot of *TaSSRP* protein

modelled with 2.5% confidence. The dimension of the 3D generated model was Model dimensions (Å): X:14.656 Y:13.262 Z:31.299 (Fig. 2B). The Ramachandran plot revealed that 70% (148 amino acid residues) of the total amino acid residues in *TaSSRP* protein, were in the core region, 19% (41 amino acids) were in the allowed region, and 5% each (11) were in the generous and outlier regions (Fig.2C). The study of *TaSSRP* will improve our understanding into its role of heat stress tolerance. *TaSSRP* can be used to develop heat-tolerant crop plants through marker-assisted breeding or a transgenic approach for climate-resilient agriculture.

Authors' contribution

Conceptualization of research (JCP); Designing of experiments (JCP, PKS, MKA); Contribution of experimental materials (JCP, PKS); Execution of field/lab experiments and data collection (MKA); Analysis of data and interpretation (MKA, KG, JCP); Preparation of the manuscript (MKA, JCP, MD, AA, PKS, VR, KG).

Supplementary materials

Supplementary Tables S1 and S2 and Supplementary Figures 1 and 2 are presented.

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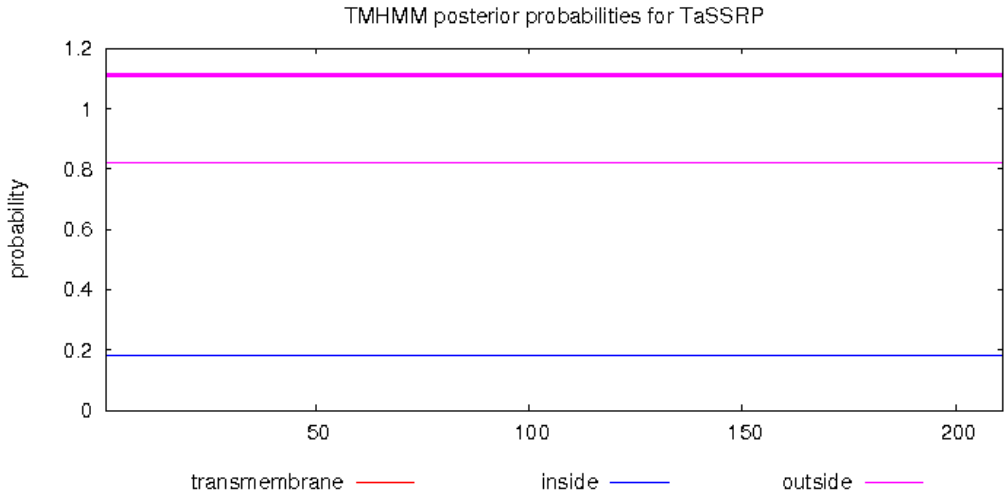
Supplementary Table S1. Subcellular location of *TaSSRP* protein, predicted by CELLO v.2.5 server

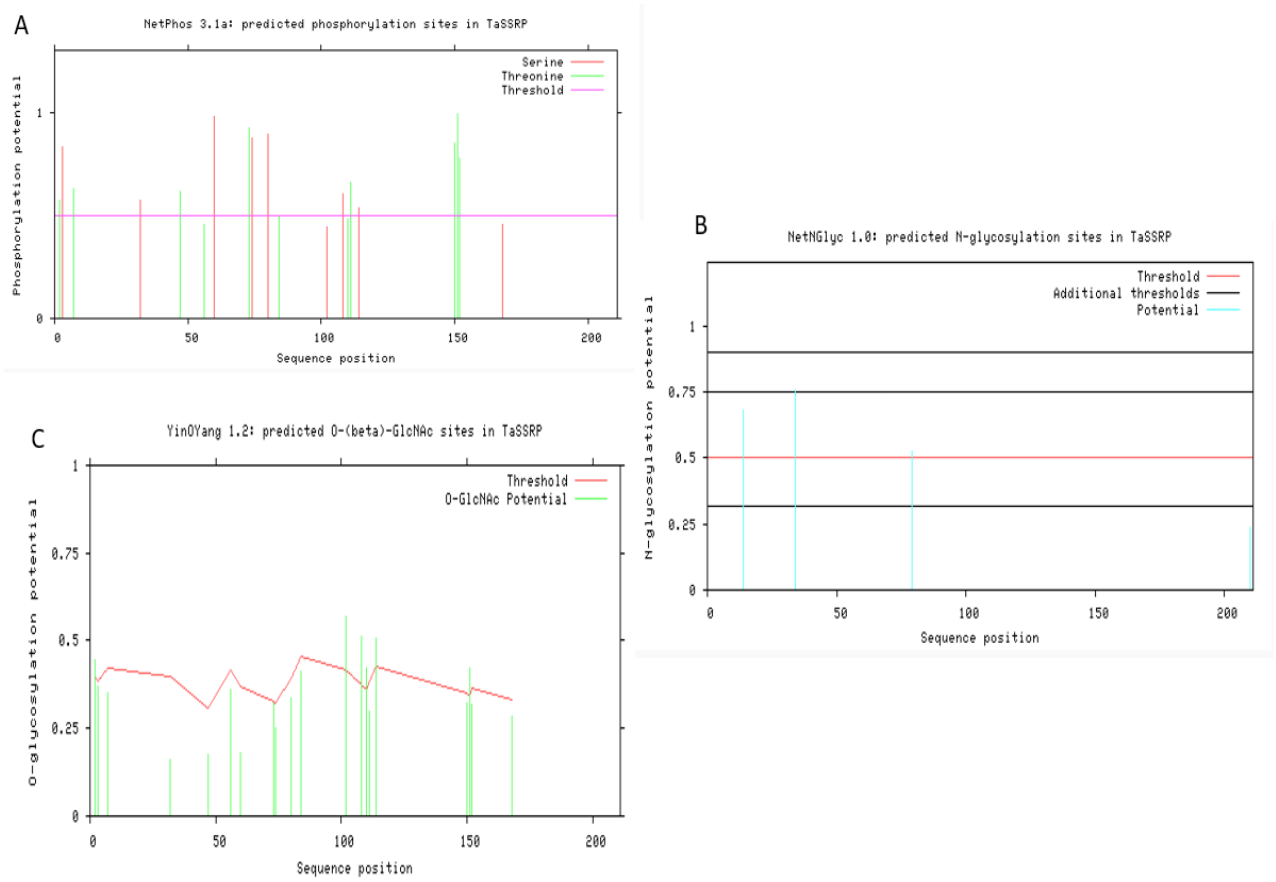
Location	Score
Cytoplasmic	2.161
Nuclear	1.107
Extracellular	0.523
Mitochondrial	0.425
Chloroplast	0.290
ER	0.188
PlasmaMembrane	0.100
Cytoskeletal	0.099
Peroxisomal	0.049
Vacuole	0.025
Golgi	0.021
Lysosomal	0.012

Supplementary Table S2. Secondary structure by GOR4

Component	Number of Amino Acids	Percentage (%)
Alpha helix (Hh)	125	59.24
3_{10} helix (Gg)	0	0
Pi helix (Ii)	0	0
Beta bridge (Bb)	0	0
Extended strand (Ee)	7	3.32
Beta turn (Tt)	0	0
Bend region (Ss)	0	0
Random coil (Cc)	79	37.44
Ambiguous states (?)	0	0
Other states	0	0



Supplementary Fig. 1. Trans-membrane helix prediction of *TaSSRP*



Supplementary Fig. 2. Post-translational modifications, (A): *TaSSRP* phosphorylated sites prediction; (B): N-linked gycosylation sites in *TaSSRP* and (C): O-linked glycosylation sites in *TaSSRP*