EXPRESSION AND BIOINFORMATIC ANALYSIS OF *ANTPS1* GENE IN NAKED OATS (*AVENA NUDA* L.)

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Abstract. Ongoing climate change has led to more extreme global temperature variations, and coupled with other environmental issues such as soil salinization, these changes pose serious challenges to future farming efforts. As such, it is vitally important both to identify crops able to resist temperature and salt stress and to explore the underlying molecular mechanisms governing such resistance in order to facilitate the selective production of crops with superior resistance traits able to persist in an increasingly harsh global environment. To that end, in this study, PCR was used to isolate the *AnTPS1* gene, which encodes trehalose 6-phosphate synthases (TPS). Real-time PCR was used to detect the expression of the gene in different tissues and under low temperature. We used a bioinformatics approach to assess key characteristics of the *AnTPS1* gene, determining its physicochemical properties, signal peptides, transmembrane structural domains, hydrophilicity, hydrophobicity, secondary structures, modules, and tertiary structures. We isolated an important abiotic stress resistance gene *AnTPS1* in naked oats. Real-time quantitative PCR analysis showed *AnTPS1* to be expressed in different tissues of naked oats, and we found that low temperature stress was sufficient to induce the upregulation of this gene. We determined that the protein encoded by *AnTPS1* gene is a soluble hydrophobic protein composed of 326 amino acids, containing signal peptides and transmembrane structures. The protein does not feature crimp spirals, instead featuring α-helices and irregular crimps are the main secondary structure elements. Together, these results provide an important biochemical foundation for the study of *AnTPS1* as a putative cold-resistance gene in naked oats, and may offer valuable insights necessary for enabling selective production of temperature-resistant crops suitable for growth in previously untenable locations.

Keywords: *naked oats, AnTPS1, gene, bioinformatics, trehalose 6-phosphate synthase (TPS), cold stress*

Introduction

Constant global climate change and rapid population increase pose significant challenges for securing a sufficient global food supply (Tai et al., 2014). There is a vital need to breed key staple crops that are able to resist abiotic stressors such as drought

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conditions, extreme cold, and highly saline soil stress (Flowers, 2004; Hu and Xiong, 2014). It is thus critically important that crops with ideal resistance traits be identified, and that the molecular mechanisms governing their resistance behaviors be fully elucidated to allow for the selective production of resistant crops capable of meeting future global food production needs.

Naked oat (*Avena nuda* L.), also known as oil wheat, jade wheat, and bell wheat, is a member of the family of Gramineae, genus Hordeum, of Chinese origin (Zhou et al., 2016). Naked oats are a cold-tolerant crop, withstanding temperatures as low as -2 to -4°C in the seedling and tillering growth stages (Liu et al., 2013). Naked oats are additionally a quick-growing, drought-resistant, and saline-alkali-resistant species. Given these key resistance characteristics, naked oats are well-suited for growth in harsh environments, and are primarily distributed in northern China and in the arid and semi-arid areas of northwest China (Ma et al., 2013). Naked oats are high in nutritional value, being rich in eight necessary amino acids, various trace elements, abundant linoleic acid, dietary fiber, and other important nutritional compounds (Wang et al., 2017). The levels of vitamin E and B in naked oats are similarly high, and the levels of protein and fat in the seeds are higher than in most food crops (Lin et al., 2011). Naked oats are primarily harvested and used for human consumption, medicine, and feeding poultry. The optimal resistance characteristics of this strain to environmental stressors make it ideal in harsh environments. Characterizing the specific molecular mechanisms underlying these resistance traits therefore offers a unique opportunity to facilitate the selective breeding or modification of strains of oats and other crops in order to allow them to better grow in non-permissive environments.

Trehalose is an important sugar molecule that is known to be vital for plant resistance to abiotic stress (Oide and Inui, 2017). Trehalose can prevent the transition of a cellular phospholipid bilayer from a liquid crystal state to the solid state, thus stabilizing proteins and other macromolecules within the bilayer and thereby enhancing the resistance of plant cells to various forms of abiotic stress (van Dijken et al., 2004). The synthesis of trehalose is catalyzed by TPS and trehalose-6-phosphate phosphatase (TPP), with the former being the rate-limiting enzyme for its production (Xu et al., 2017). The introduction of fusion genes for TPS and TPP from *Escherichia coli* and yeast into transgenic tobacco, potato, rice and *Arabidopsis* plants has been found to increase resistance to abiotic stress such as drought tolerance among these transgenic plants (Delorge et al., 2015). These studies thus indicate the TPS gene plays an important role in enhancing plant stress resistance, making it an important target of research aimed at improving the resistance characteristics of crops. This has led to extensive efforts to better understand the role of trehalose in the context of abiotic stress resistance, and as such TPS gene sequences have been isolated and validated from herbaceous plants such as *Arabidopsis thaliana* in recent years (Xu et al., 2017). Whether particular species of plants possess unique TPS genes with potentially advantageous properties remains to be determined, and at present there are no reports on the characteristics of TPS in naked

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oats. As such, in the present study we used a bioinformatics approach to assess the properties of the *AnTPS1* gene in naked oats, exploring its physicochemical properties, signal peptides, transmembrane structural domains, hydrophilicity, hydrophobicity, secondary structures, modules, and tertiary structures. Through these efforts, this study aimed to provide a theoretical basis both for improving naked oats yield and quality, and to more broadly produce information that may be of value for the selective production of crops with advantageous abiotic stress resistant traits.

Materials and methods

Materials and cold treatment

Naked oats cultivar Jinyan 17 was used in this study. Seeds were sterilized through incubation for 1 min in 75% ethanol, and thoroughly being washed with sterile water. The seeds were germinated in soil in pots at 20°C under long-day conditions (16 h of cool white fluorescent light, photon flux of 70 umol m^{-2} s^{-1}). 50 Seedlings at the four-leaf stage were subjected to cold stress. 50 Seedlings in the control group were grown at 20°C continuously. To induce cold stress, seedlings were transferred to an artificial climate box at 4°C under the same light and photoperiodic conditions. The leaves were then treated with cold temperature at 4°C for 0 h, 1 h, 3 h, 6 h, 12 h, 24 h (Indeok, 2016). This stressor procedure was conducted in triplicate.

AnTPS1 gene expression analysis

Total RNA was extracted from plants using the RNA Plant Plus Reagent Kit (Tiangen Biochemical Technology Co., Ltd.) and was reverse transcribed into cDNA using the FastQuant RT Kit. The cDNA of *AnTPS1* was amplified by PCR and sequenced. The primer sequences were: AnTPS1-F1 (5'- CATCACGCTG CTCTCTCTAC -3') and AnTPS1-R1 (5'- GAGGTCCGACACTTGCGTT -3'). The relative expression of *AnTPS1* was then detected by real-time fluorescent quantitative PCR (Guo et al., 2015; Reem et al., 2017). The cDNA derived from root, stem, and leaves of plants exposed to temperature stress treatment were used for all qPCR reactions. The primer sequences for *AnTPS1* were: AnTPS1-F2 (5'- TCCTATGCTGTGTCCCAATC -3') and AnTPS1-R2 (5'- CAGACCAGTGAACAATAGGG -3'). *Actin* served as an internal reference gene; the primer sequences for actin were: Actin-F (5' TATTGCTTTCTCCTGCTGTC-3') and Actin-R (5'-CTATGTATCCTGGTATTGCG-3'). The qPCR reaction was conducted under the following conditions: pre-denaturation at 95°C for 3 min; denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, 30 cycles. A melting curve was then constructed via the system default procedure. All samples were run in triplicate.

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Biological information analysis of AnTPS1

A comprehensive bioinformatics analysis was conducted using tools including the NCBI, PDB, and ExPASy databases and further online ones (*Table 1*) (Lu et al., 2018), in order to predict and analyze characteristics such as conserved domains, the physicochemical properties of the primary structure, signal peptides, transmembrane structural domains, subcellular localization, hydrophilicity and hydrophobicity, secondary structure, modules, and tertiary structures (Mao et al., 2018). Briefly, bioinformatics tools were used as follows: CD Search was used for conservative domain prediction; the ExPASy Protparam tool was used to predict the physiochemical properties of the encoded protein; the ExPASy Protscale tool was used for hydrophobicity/hydrophilicity predictions; SignalP4.1 Server was used for signal peptide predictions; TMHMM Server v.2.0 was used for predicting transmembrane regions; SOSUI was used to predict protein solubility; SOMPA was used for secondary structure predictions; CILS was used for crimp spiral analyses; SWISS-MODEL was used for tertiary structure modeling.

Table 1. *Web tools for predicting protein structure and function*

Application website	Website	Website usage
BLAST	https://blast.ncbi.nlm.nih.gov/Blast.cgi	Sequence download
CD search	http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi	Conservative domain prediction
Protparam	http://www.expasy.org/tools/protparam.html	Physicochemical properties prediction
SignalP4.1Server	http://www.cbs.dtu.dk/services/SignalP	Signal peptide prediction
TMHMM Server v. 2.0	http://www.cbs.dtu.dk/services/TMHMM/	Transmembrane region prediction
SOSUI	http://harrier.nagahama-i-bio.ac.jp/sosui/sosui_submit.htm	Soluble protein prediction
ProtScale	http://ca.expasy.org/tools/protscale.html	Hydrophobic and hydrophilic prediction
SOMPA	https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html	Secondary structure prediction
COILS	http://www.ch.embnet.org/software/COILS_form.html	Crimp spiral analysis
SWISS-MODEL	https://www.swissmodel.expasy.org/	Tertiary structure modeling
3DLigandSite	http://www.sbg.bio.ic.ac.uk/~3dligandsite/	Ligand binding site prediction

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Results and analysis

Expression analysis of the AnTPS1 gene

The cDNA of *AnTPS1* was amplified by PCR (*Fig. 1*) and then sequenced (*Fig. 2*). To assess the expression and relevance *AnTPS1* to cold stress in naked oats, we used real-time qPCR to assess its expression within various parts of the naked oats plant. Results showed the *AnTPS1* gene to be most abundantly expressed in the leaves of naked oats, with lower levels in stems, and limited expression in roots (*Fig. 3*). Under low temperature cold stress conditions, the expression of *AnTPS1* changed significantly. The gene expression was significantly increased at 3 h and 6 h. After 6 h, the expression level reached its maximum – roughly twice that of the control plants - and then decreased significantly (*Fig. 4*).

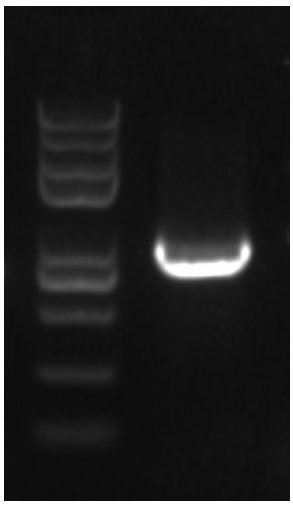

Figure 1. *The AnTPS1 fragment amplified by PCR*

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1     ATGGAGAAAT  CCTGTCCAGC  AGCCACCGCC  GGCCTAGGAG  AGGAACTCTT  CATGCCAGCA
61    TGGGCTCCTA  TGCTGTGTCC  CAATCCCAGG  CGAGCACGGA  AGGAGCCGTT  AAGCACGATG
121   TCGTTCGACA  GGGACTACTC  GTTCCGAATG  AGTACCTTCT  GGTCGTGGAG  GATCCATCTG
181   TGCCTTTTTT  TTGAGCTTGT  TTCCTGCATA  ATTCTACCAT  TCAGATGGAT  GTGCGATCGC
241   TTTGCCCCCA  GCAGATCAAT  CCGAGAACCA  CGCCCGAACT  TGTCTTTGAG  CTTCTTTGAA
301   GATGACCGCG  GTTACATGGA  TTATCCTTTT  CGGAAGAGGA  CTCCCGAGTA  TGTTGAAAGA
361   ATTGCTTGGA  GTGAATGGAT  GATGCTTCTT  ATTTTTGTGA  TGGTGGAAGG  CTGGTACATG
421   ATCAAGGATG  CATGCTGGAT  CGAGGATGCC  TGGTCAACTG  CTATTATGTT  TTGTCCTTAT
481   ATTCTTCAGG  GGCGTCTGAA  GCACATCAAA  AGCCATATTT  TCGGAAAACC  AAGGATGGTT
541   CCGACGGAAA  CAATTGCGGC  TTTTGGTTCT  TCTTCAACTG  GCAGTGGTCG  CTTTATAATG
601   GACTCTGGTG  CCACTTCACA  TTTTGTTGGT  AATGCCAGTA  TGCTGCAGGA  CATCATATAT
661   TTTCCTCTTG  AGCAGCGTCA  GCTTGTTACC  TTAGCAGATG  GCTTCTGTCT  TCCCATTGTC
721   GGCATTGGCA  CCCTATTGTT  CACTGGTCTG  AGCGAGTGTG  GGAGCTATAC  TCGGTCCTAC
781   AGAGTCCCCA  ATGTCCGCTA  CGTGCGTGGC  CTTGCGATGA  ACGTGATATC  TGTCGGCCAG
841   CTTGACAATG  AGCATGGCTT  GTGCAGCGCC  TTCCACAGCG  AGAAATGCGA  GATTATGGCT
901   GACAGAACCG  TCATTGGGCA  AGCTGTTCTT  GTGAACCGCT  TGTACGAGGT  GGTCTACCTC
961   CATGTCCCAC  GAGTGTCGTA  G
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Figure 2. *Sequence of the AnTPS1 gene*

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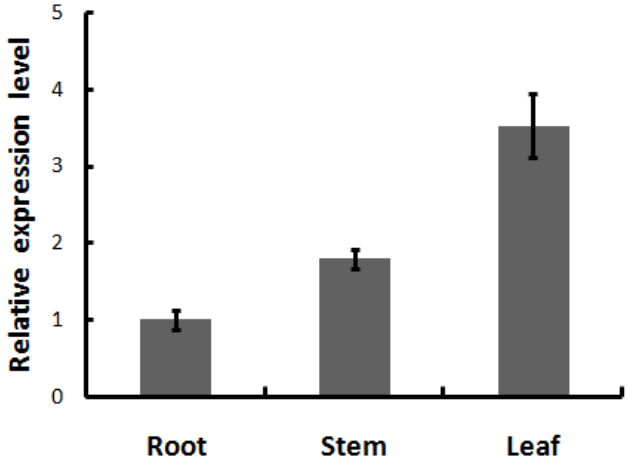

Figure 3. *Relative expression levels of AnTPS1 in different tissues*

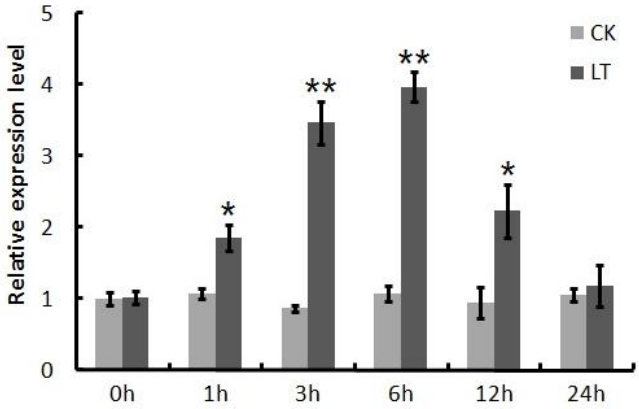

Figure 4. *Relative expression levels of AnTPS1 at different times under low temperature treatment. Note: CK represents control, LT represents low temperature treatment*

Analysis of AnTPS1 primary structure and physicochemical properties

We predicted and analyzed the amino acid composition and physicochemical properties of AnTPS1, which encodes TPS, using the ProtParam tool available from ExPASy. We found *AnTPS1* to encode a 326 amino acid protein enriched in serine and leucine (8.6% each), and with a low glutamine content (1.8%). The protein formula for AnTPS1 was $C_{1694}H_{2592}N_{448}O_{458}S_{28}$, with 5220 total atoms and a relative molecular weight of 37459.62 Da. The total number of negatively charged amino acid residues (Asp and Glu) and positively charged amino acid residues (Arg and Lys) were 32 and 34, respectively. The theoretical isoelectric point (pI) of this protein was 7.99, leaning towards alkalinity. The extinction coefficients at 280 nm for this protein were 68005 $M^{-1} \cdot cm^{-1}$ when assuming all cysteine residues were from cysteine, and

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67380 $M^{-1} \cdot cm^{-1}$ when assuming none were. Based on an N-terminal methionine, the estimated half-lives of this protein in mammalian reticular cells (*in vitro*), yeast (*in vivo*), and colibacillus (*in vivo*) were 30 hours, more than 20 hours, and more than 10 hours, respectively. The instability coefficient was 82.55, classifying AnTPS1 as an unstable protein. The fat coefficient of AnTPS1 was 51.90. The total average of hydrophilicity of this protein was 0.052, classifying *AnTPS1* as a hydrophobic protein.

AnTPS1 protein signal peptide, transmembrane domain, and hydrophilicity predictions

Through the measurement of protein shearing sites, signal peptide predictions can categorize the functional domains of proteins and determine their likely subcellular localization. An assessment of AnTPS1 using the SignalP4.1Server yielded a cleavage site score (C-score) of 0.168, a synthetic shearing site score (Y-score) of 0.167, and a signal peptide score (S-score) of 0.248 (*Fig. 5*). These results were consistent with AnTPS1 being a secretory protein containing a signal peptide, suggesting that the resultant TPS protein may be involved with transmembrane transport within cells.

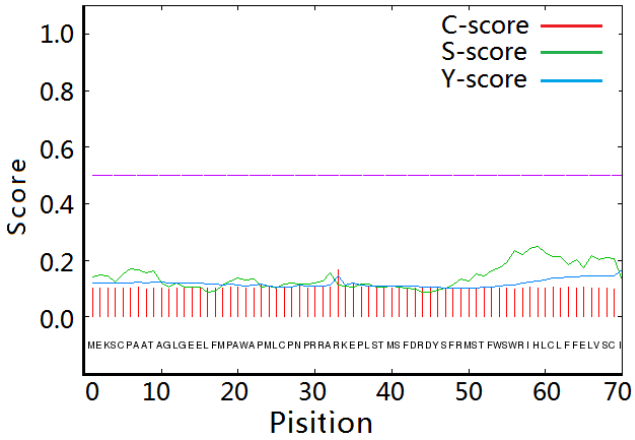

Figure 5. *Signal peptide analysis of the AnTPS1 protein by SignalP 4.1*

Membrane proteins may have extracellular, transmembrane and intracellular domains (Fraihat et al., 2018; Radwan et al., 2018; Saeed et al., 2018; Rajagopalan et al., 2018). Transmembrane regions generally are α-helical structures with hydrophobic amino acids embedded in the cytoplasmic membrane. A transmembrane structure analysis of the *AnTPS1* protein using the TMHMM Server v2.0 revealed that the encoded protein has a transmembrane helix (TMH) with a TMH value of 57.31 (*Fig. 6*). The value of the non-transmembrane helical region (ExpAA) of this protein was 6.41447, consistent with it being transmembrane protein. The probability of the N-terminal domain of the protein locating inside the membrane was 0.2999, and this sequence is typically located outside the membrane, further suggesting that the protein encoded by *AnTPS1* is a

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transmembrane protein. An analysis of the solubility of the *AnTPS1*-encoded protein via SOSUI revealed that this protein was a membrane protein with two transmembrane helices and a total length of 326 amino acids – slightly longer than the initial prediction. This protein was predicted to be soluble, with an average hydrophobicity of 0.037160.

Amino acid hydrophilicity is the primary force driving protein folding, and it can thus help in the evaluation of secondary structural features such as transmembrane helices and amino acid surface distributions within a given protein (Woldesemayat et al., 2017). The hydrophilicity of the naked oats *AnTPS1* amino acid sequence was predicted using the ExPASY ProtScale tool (*Fig. 7*), revealing the 131st amino acid in the peptide chain to be the most hydrophobic, with a positive score of 3.222; while amino acids 31 and 32 were the most hydrophilic, with a negative score of -2.867. *Figure 4* illustrates that the N-terminal domain of this protein is negative (hydrophilic), while the C-terminal domain is positive (hydrophobic), with fewer hydrophilic amino acids in the overall peptide chain than hydrophobic ones. This suggests *AnTPS1* is a soluble hydrophobic protein.

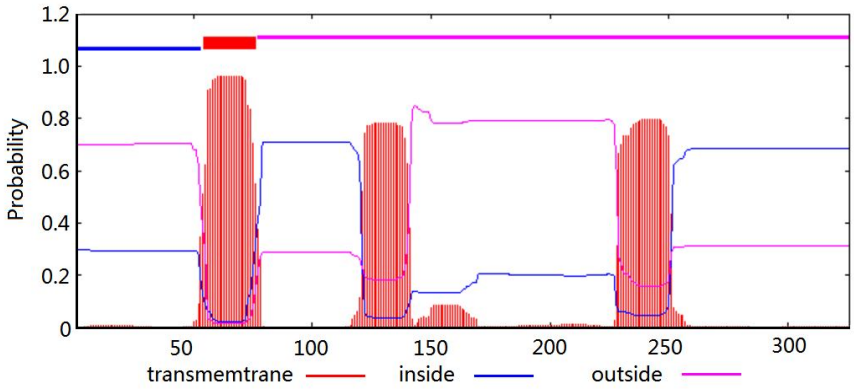

Figure 6. *Transmembrane analysis of the AnTPS1 protein by TMHMM*

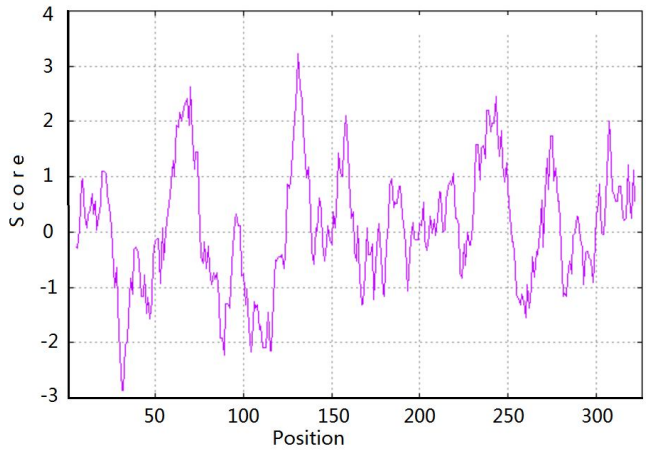

Figure 7. *Prediction of hydrophilicity of the AnTPS1 protein by ProtScale*

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Prediction and analysis of AnTPS1 protein secondary structure and modules

The secondary structure of the AnTPS1 protein was assessed using the SOMPA online prediction program, which predicted 113 amino acid residues in the protein to be, accounting for 34.66% of the overall peptide chain. In total, 63 amino acid residues had an extended chain structure (19.33% of the overall peptide chain), 29 amino acid residues had a β-rotation structure (8.90% of the overall peptide chain), and 121 amino acid residues had an irregular curl structure (37.12% of the overall peptide chain) (*Fig. 8*). The α-helix and irregular crimp were the main structural components of the AnTPS1 protein, contributing to its structural stability (Geourjon and Deléage, 1995). The PredictProtein tool was used to predict the presence of disulfide bonds, none of which were predicted to reside within the resultant protein. Functional sites were analyzed using the PROSITE tool, identifying 11 phosphorylation sites, 2 glycosylation sites, and 5 acylation sites.

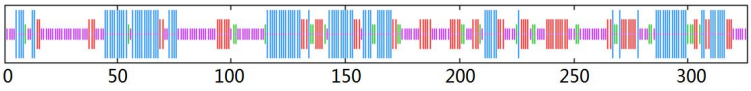

Figure 8. *Analysis of the secondary structure of the AnTPS1 protein by SOMPA. Note: blue represents α-helical, red represents extended strand, green represents β-rotation, purple represents irregular curl*

AnTPS1 protein tertiary structure modeling and binding site prediction

SWISS-MODEL was utilized for homology modeling of this predicted protein (using PyMOL view) (*Fig. 9*), while a Ramachandran evaluation in MolProbity software was employed to evaluate the tertiary structure of the AnTPS1-encoded protein as previously described (Liu et al., 2017). Modeling by the SWISS-MODEL server is based on the A chain of PDB ID 3d79.1, with a sequence consistency value of 20.41%. Ramachandran plot-based evaluation determined that 93.64% (103/126) of amino acid sites are located in the favored region, while 100% (126/126) are located in the permitted region, indicating that the tertiary structure model is accurate and reliable.

Figure 9. *Tertiary structure of the AnTPS1 protein predicted by SWISS-MODEL*

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Discussion

Trehalose is a stress metabolite, only accumulating in plants under conditions of stress such as drought, extreme temperatures, and high salinity (Chary et al., 2008). The accumulation of trehalose enhances the protection of proteins, enzymes, and cell membranes, preserving cellular and organismal viability. Trehalose is degraded when the noxious environmental conditions subside (Lunn et al., 2014). The TPS gene is essential for the synthesis of trehalose in plants. Introduction of the exogenous TPS allows for the synthesis of trehalose 6-phosphate (T6P), which is dephosphorylated to form trehalose by a wide range of non-specific phosphatases, making TPS the rate-limiting enzyme for trehalose accumulation (Cai et al., 2009).

TPS is important for glycometabolism, embryonic development, and the response to abiotic stress, making it essential for overall crop development. The TPS gene family has been evaluated structurally, functionally and evolutionarily in rice, Arabidopsis thaliana, poplar, and cotton (Tang et al., 2018). However, similar studies have not been conducted to date in naked oats, despite the notable hardiness of this species and its effective stress resistance, making it an important species in which to study such a key abiotic stress resistance gene. Bioinformatics allow for the detailed prediction and analysis of predicted proteins encoded by particular gene sequences, offering potential insights into the structure and function thereof. Our assessment of the peptide sequence of the AnTPS1-encoded protein revealed the ORF to encode for 326 amino acids without multiple domains. The N-terminal TPS (T6P synthase) domain catalyzes the production of T6P using glucose-6-phosphate and UDP-glucose as substrates, whereas the C-terminal TPP domaindephosphorylates T6P, generating trehalose (Matsuda et al., 2015). The protein formula of AnTPS1 is C1694H2592N448O458S28, with a relative molecular weight of 37459.62 Da and a predicted isoelectric point (pI) of 5.75. The protein is positively charged and leans towards alkalinity. It is a soluble hydrophobic protein containing a signal peptide and transmembrane helix. The α-helix and irregular crimp are the main structural components of the AnTPS1 protein. In a previous study by Tang et al. (2018) in N. lugens, three TPS genes were cloned, and their protein secondary structures contained similar components to those identified in the present study, with α-helices, β-sheets, and random coils. We further verified that the AnTPS1 protein had a signal peptide, consistent with the results of the transmembrane structure analysis. Subcellular localization revealed the AnTPS1 protein to be potentially located in the cytoplasm, which is consistent with a finding by Xu et al. (2017) which revealed that the majority of TPS in Solanum tuberosum may be located in the cytoplasm, with only limited quantities located in the plasma membrane or nucleus. This variation in localization may be dynamic, potentially having a relationship with stress response behavior, although further microscopy-based studies would be needed to verify this hypothesis. The localization of a given protein is closely related to its functional involvement. In assessing the functional domains of the protein encoded by *AnTPS1*, we

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identified 11 phosphorylation sites, 2 glycosylation sites and 5 acylation sites without crimp helix domain in the protein. Together, these predictive bioinformatics analyses confirmed that the protein encoded by AnTPS1 is consistent with other published TPS protein sequences and structures, suggesting that such structural homology is likely accompanied by functional homology as well in the context of stress responses.

Analysis of the expression of *AnTPS1* in different tissues of naked oats revealed that this gene is expressed at the highest level in leaves and at the lowest level in roots. This differential expression may be due to differences in developmental differentiation, spatial location, function, metabolic activity, and environmental conditions among these different organs and tissues (Jin et al., 2016). We found that the *AnTPS1* gene was up-regulated by cold temperature stress, indicating it to have an expression pattern consistent with the abiotic stress response to cold and potentially to other factors as well. Thus, *AnTPS1* may play a role in the resistance of naked oats to low temperature stress, as has been found in other studies. Indeed, transgenic plants expressing TPS have increased tolerance to stress (Mu et al., 2016). In Arabidopsis plants, transgenic TPS expression was shown to substantially increase resistance to extreme stress without disrupting normal cellular morphology (Miranda et al., 2007). Despite these promising results, some authors have raised concerns that trehalose expression may be advantageous under stress conditions, but may become deleterious over time, making plants more susceptible to certain plant pathogens and potentially altering growth kinetics (Fernandez et al., 2010). Interestingly, we found that the expression of *AnTPS1* increased substantially under cold stress conditions, before declining at later time points, potentially suggesting that the *AnTPS1* gene from naked oats may be regulated in an optimal fashion that allows it to be acutely expressed as a part of the early stress response, before decreasing expression levels over time to reduce potential adverse outcomes of prolonged expression. Selective expression of comparably regulated proteins in other crops could feasibly offer optimal resistance characteristics without disrupting normal morphology, although more work will be needed to confirm such a hypothesis. In naked oats, the intermediate metabolite T6P catalyzed by the TPS domain may participate in the response to cold stress by acting as signaling molecule, or by catalyzing the production of trehalose which is important for the osmotic regulation of naked oats in response to abiotic stress.

There are limitations to the present study that should be considered. For one, the bioinformatics-based predictive approaches are by their very nature uncertain. While all tools were used as intended, inherent uncertainties in the resultant predicted *AnTPS1* protein structure and localization can result in apparent inconsistencies in its characteristics. Further molecular approaches will be needed to therefore validate its subcellular localization and expression patterns, and to determine whether its localization changes depending on the functional context. In addition, while our *in vivo* assessment of *AnTPS1* expression revealed patterns of expression consistent with a stress response, formal knockout experiments will be needed to fully validate the role of

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this gene in abiotic stress responses in naked oats. Given that naked oats are well-adapted to growth under adverse conditions, and that we observed time-dependent expression of this trehalose-producing gene in response to stress, it is possible that further study of the optimal regulation and expression patterns of this gene will yield further fundamental insights into optimal stress resistance strategies suitable for improving global crop yields.

Conclusion

Ongoing environmental changes and a rapidly increasing global population both necessitate the growth of selectively bred or transgenic crops capable of withstanding serious environmental stress conditions. In this study, we analyzed the expression and bioinformatics analysis of *AnTPS1* gene. *AnTPS1* was upregulated by low temperature. The expression of *AnTPS1* in naked oats represents an important step towards understanding the molecular mechanisms governing the responses of plants to abiotic stressors. While further study will be necessary to validate these results using genetic modifications and molecular imaging approaches, this work represents an important step towards the production of robust and resistant plant species.

Contributions. Wenying Liu and Feng Li conceived and designed the study. Rui Liu, Lizhen Liu, and Yongfang Zhang performed the experiments. Gang Li and Hongxian Tian provided the seeds. Wenying Liu and Jianxia Liu wrote the paper. Runmei Wang, Feng Zhou, and Dongxu Zhang reviewed and edited the manuscript. All authors read and approved the manuscript.

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