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SnRK2 acts within an intricate network that links sucrose metabolic and stress signaling in wheat

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Drought, salinity and low temperature are major environmental factors that influence plant growth and development, and eventually limit crop yield and quality. To survive adverse stresses, plants have developed complex signaling networks to perceive external stimuli, and then manifest adaptive responses at molecular and physiological levels. Sucrose non-fermenting1-related protein kinase 2 (SnRK2) plays a critical role in plant sugar signaling via phosphorylation, while knowledge of specific functions of *SnRK2s* in wheat is still undiscovered. In this paper, we reviewed our recent studies on wheat SnRK2 members, *TaSnRK2.4*, *TaSnRK2.7* and *TaSnRK2.8*, involved in abiotic stress responses. The results suggest that the three wheat kinases participate in sugar metabolic and stress signaling in wheat. Furthermore, we compare their distinct transcript levels in various tissues, expression patterns under diverse stress conditions and functions in transgenic Arabidopsis.

The sucrose non-fermenting-1 (SNF1) protein kinase family comprises SNF1 itself in yeast, the AMP-activated protein kinases (AMPK) in mammals and the SNF1-related protein kinases (SnRKs) in plants. The plant SnRKs are divided into three subfamilies (SnRK1, SnRK2 and SnRK3) based on the amino acid sequence identity and expression patterns. SnRK2 is a type of serine/threonine protein kinase, including two typical domains, viz., an N-terminal catalytic domain and a C-terminal regulatory region, characterized by the presence of a short acidic patch. Based on the C-terminal acidic patch, the SnRK2 family is divided into two distinct subclasses, SnRK2a and SnRK2b. The acidic patch of SnRK2a is rich in aspartic acid residues, while that of SnRK2b enriches in glutamic acid residues.[1,2] Increasing evidence shows that *SnRK2s* act within an intricate network that links metabolic and stress signaling in plants.[1-4] Despite these important functions in plants, knowledge of specific functions of *SnRK2s* in wheat is fragmentary and the molecular mechanism of their activation is still enigmatic. In this review, we highlight the current view on the role of SnRK2 in plant carbohydrate metabolic and stress signaling pathways.

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PKABA1, the first cloned SnRK2 member in wheat, was induced by ABA and dehydration stress, and it repressed the activities of gibberellic acid-inducible promoters when transiently overexpressed in barley aleurone layers.[5,6] In our recent studies, three wheat SnRK2 members, *TaSnRK2.4*, *TaSnRK2.7* and *TaSnRK2.8* were cloned and characterized. Experimental evidence supported they were extensive regulatory factors in carbohydrate metabolism and stress signal transduction.[7-9] Here, a phylogenetic tree was constructed with putative amino acid sequences of PKABA1, TaSnRK2.4, TaSnRK2.7, TaSnRK2.8 and SnRK2 family members in Arabidopsis, rice and maize (**Fig. 1**). TaSnRK2.4 and TaSnRK2.7 were clustered in the SnRK2a subclass and PKABA1 and TaSnRK2.8 fell into the SnRK2b subclass. Furthermore, the counterparts from Arabidopsis, rice and maize were clustered in the same clades, implying the emergence of SnRK2 occurred before the separation of monocots and dicots.

Global Regulator of Carbohydrate Metabolism

Expression pattern is a direct indicator of a gene's involvement in developmental or differential events. In Arabidopsis, *AtSRK2.8/AtSRK2C* was identified as a root-specific protein kinase and *AtSRK2.6/AtSRK2E/OST1* was confirmed to play a pivotal role in stomatal closure in leaves.[10,11] Gene expression patterns in various wheat tissues showed that *TaSnRK2.4*, *TaSnRK2.7* and *TaSnRK2.8* were constitutively expressing genes; the highest expression of *TaSnRK2.4* occurred in booting spindle, while that of *TaSnRK2.7* and *TaSnRK2.8* occurred in root. Moreover, all three proteins were present in the cell membrane, cytoplasm and nucleus. Thus, SnRK2 kinases existed extensively in plant cells and tissues.

It is well documented that yeast SNF1-kinase and mammalian AMPK have key roles in sugar metabolism.[1,2] Similarly, our recently results showed that *TaSnRK2.7* was mapped on chromosome 2AL with the flanking markers WMC179.4 and WMC401,[12] which were co-located in the same or adjacent chromosome intervals with QTLs for phosphorus utilization efficiency[13] and accumulation efficiency of stem water-soluble carbohydrates.[14] To unravel the roles of the SnRK2 in the regulation of carbohydrate and energy metabolism, the three *SnRK2s* were transferred to Arabidopsis, respectively and the significant lower total soluble carbohydrate in transgenic Arabidopsis was identified. The results suggested that SnRK2 was involved in

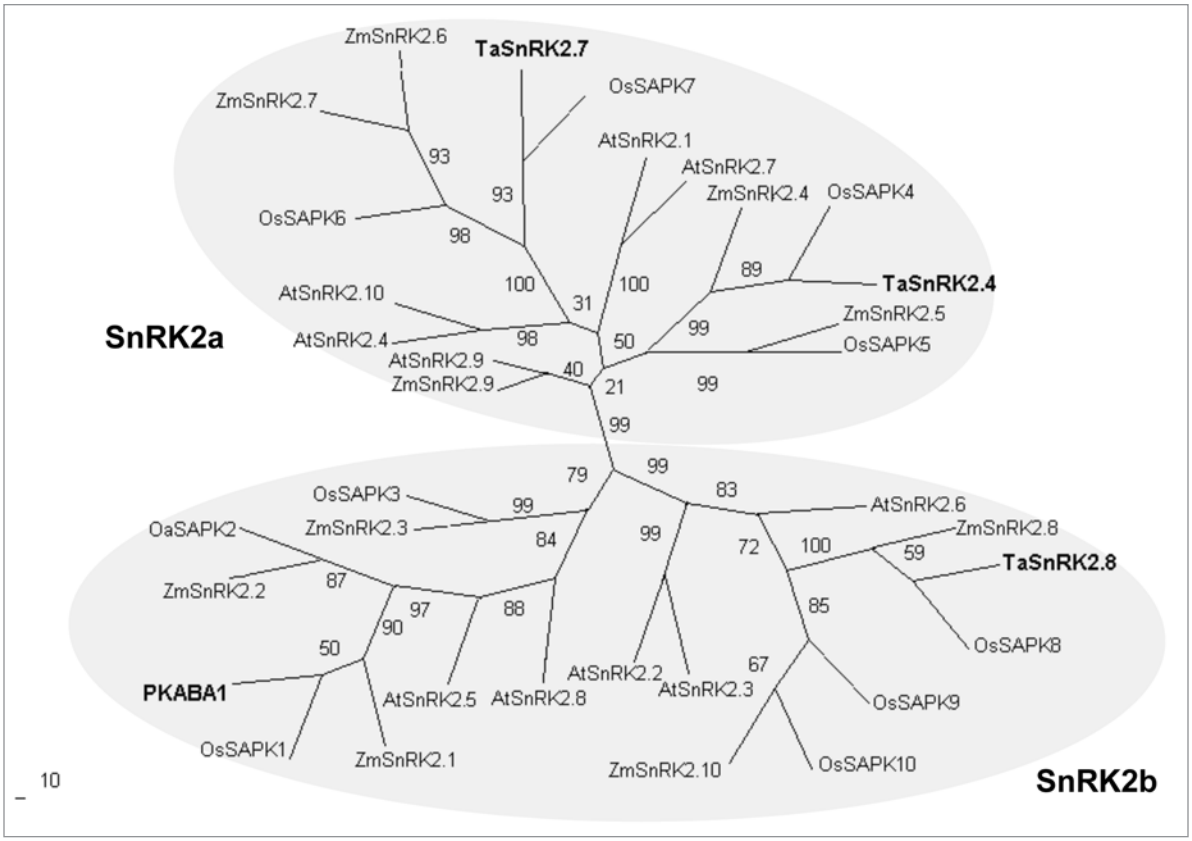

Figure 1. Phylogenetic tree of four wheat SnRK2 members and SnRK2s from other plant species. Two distinct isoform groups are presented in grey. Ta, *Triticum aestivum*; Os, *Oryza sativa*; At, *Arabidopsis thaliana*; Zm, *Zea mays*. The phylogenetic tree was constructed with the PHYLIP 3.68 package; bootstrap values are in percentage.

carbohydrate metabolism. As a result, it could function in plant growth and development, such as overexpression of *TaSnRK2.4* in Arabidopsis resulted in the delayed seedling establishment and longer primary roots, and overexpressing *TaSnRK2.7* and *TaSnRK2.8* leaded to improved root growth and development, respectively.

Pivotal Factor in Stress Signal Transduction

Besides the prime carbon and energy source to plant growth and development, sugars can complement and interact with various hormones and growth factors signaling mechanisms to regulate metabolism and stress-resistance in complex systems. Recently, the pivotal roles of sugars in plant growth and development, and key players in sugar signaling network have been uncovered.[15-17] As an integral component of the sugar signaling pathway, the plant SNF1 complex has been studied intensively. Currently, evidence suggested that the phosphatase PP2C acted as a constitutive negative regulator of SnRK2 kinases in the absence of the phytohormone abscisic acid (ABA) and the presence of ABA could enable the PYR/PYL/RCAR proteins to bind to and repress PP2C. Sequestration of PP2C permitted the auto-activation of SnRK2 kinases, which could phosphorylate downstream transcription factors (ABF/AREB) and facilitate transcription of ABA-responsive genes.[18-20] These studies were focused on the plant hormone ABA, which was often recruited as the primary signal for increasing the transcription levels of the stress responsive genes, while some SnRK2 members might participate in ABA-independent signal transduction pathways.[21,22] Until now, little is known in detail about its role in plant ABA-independent stress signaling.

In our research, although all the three members, *TaSnRK2.4*, *TaSnRK2.7* and *TaSnRK2.8*, were involved in response to PEG, NaCl and cold stresses in wheat, they exhibited distinct expression patterns. Moreover, *TaSnRK2.4* and *TaSnRK2.8* could be induced by ABA treatment, whereas *TaSnRK2.7* might be involved in ABA-independent pathway. Function analysis indicated that all plants overexpressing *TaSnRK2.4*, *TaSnRK2.7* and *TaSnRK2.8* exhibited the enhanced resistance to multi-abiotic stresses through increasing osmotic adjustment ability, promoting photosynthetic capability and strengthening seedling roots. These supported that wheat SnRK2 members might be involved in different stress signal pathways.

To elucidate the molecular mechanism of *SnRK2s* in stress response, the expression levels of the genes participated in ABA biosynthesis and signaling or those involved in stress protection were investigated in transgenic Arabidopsis. As expected, the transcript levels of ABA biosynthesis genes (*ABA1*, *ABA2*), ABA signaling genes (*ABI3*, *ABI4*, *ABI5*), stress-responsive genes including two ABA-dependent genes (*RD29A*, *RD29B*)

and three ABA-independent genes (*CBF1*, *CBF2*, *CBF3*) were generally higher in transgenic *TaSnRK2.8* plants than control plants under both normal and stress conditions. Intriguingly, the findings suggested that *TaSnRK2.8* may act on the upstream regulators of these genes in stress tolerance, and thus directly or indirectly involved in ABA-dependent and ABA-independent signaling networks.

Conclusions and Perspectives

The results presented here demonstrate that (1) wheat SnRK2s were multifunctional regulatory factors, acted within an intricate network which linked metabolic and stress signaling in plant. Overexpression of *TaSnRK2s* in Arabidopsis remarkably enhances tolerance to drought, salt and cold stresses without growth retardation. Therefore, there is a potential to utilize them in the stress-tolerance improvement in crops. (2) Individual members of SnRK2 had evolved specifically for stress signaling and acquired distinct regulatory properties. (3) Each member might be involved in multiple signaling pathways. In future investigations, it will be interesting to determine the biochemical properties and precise roles of wheat SnRK2 in metabolic and stress signaling to further advance the understanding of its adaptive mechanisms under stress conditions.

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