Review Article

Sucrose non-fermenting 1-related protein kinase 2 (SnRK2) : a family of protein kinases involved in hyperosmotic stress signaling

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ABSTRACT

Our understanding of plant adaptation to abiotic stresses, which include drought, salinity, non-optimal temperatures and poor soil nutrition, is limited, although significant strides have been made in identifying some of the gene players and signaling partners. Several protein kinases get activated in plants in response to osmotic stress and the stress hormone abscisic acid (ABA). Among these is a superfamily of sucrose non-fermenting protein kinase genes (SnRK2). This review focuses on the developments related to the activity, substrates, interacting proteins and gene regulation of SnRK2 gene family members. Reversible phosphorylation as a crucial regulatory mechanism turns out to be a rule rather than an exception in plant responses to abiotic stress. Nine out of thirteen bZIP transcription factors (ABI5/ABF/AREB family) share the recognition motif, R-Q-X-S/T, suggesting that likely SnRK2 kinases have a major role in regulating gene expression during hyperosmotic stress. **[Physiol. Mol. Biol. Plants 2008; 14(1&2) : 91-100]** *E-mail : autar.mattoo@ars.usda.gov*

Key words : *Protein kinases; ABA signaling; phosphatases; phosphorylation; environmental stresses.*

Abbreviations : *ABRE, ABA responsive element (CACGTGGC); G box, core sequence ACGT in CCACGTGG, TGACGTGG; CE, coupling elements (CE1: TGCCACCGG; CE3: ACGCGTGTC); ABF, ABRE binding factors; bZIP, protein containing a basic amino acid enriched region adjacent to a leucine zipper*

Plants are sessile organisms and therefore to survive must adapt to a number of environmental extremes they face continuously. These environmental challenges include cold, heat, high irradiances, drought, salinity, and rising atmospheric CO_2 levels (in part contributed by global warming). Most of these abiotic stresses lead to major agricultural losses due to poor crop growth and quality (Zelitch, 1982; Neumann, 1997; Mattoo *et al*., 2000; Basra, 2001; Serraj and Sinclair, 2002; Long *et al*., 2006). One common feature across several of these stresses seems to be osmotic in nature. Osmotic stress results in a change in state, activity or expression of genes, cation/anion movements, protein secretion and enzymes of affected cells. The stimulus is provided by the change in the concentration of signal molecules in extra-cellular milieu compared to that present intracellularly, or vice versa. Under these conditions, cells may shrink in size (hyper-osmotic stress) or swell (hypo-osmotic stress) and lead to ionic imbalance, DNA and protein damage, cell cycle arrest and finally cell death. Salinity and drought cause hyper-osmotic stress (Neumann, 1997; Serraj and Sinclair, 2002). To survive such adverse conditions, plants have developed sophisticated response pathways, first to sense the change in their environment and then transduce the signal to cellular machinery such that physiological mechanisms are put into motion to adjust to the environmental extreme (Finkelstein *et al*., 2002; Chinusamy *et al*., 2004; Cao *et al*., 2007). Plants that are efficient in sustaining such response pathways for a longer time are stress tolerant and those that can not eventually die.

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Abscisic acid (ABA) and Stress

Phytohormone abscisic acid (ABA) plays a major role during plant exposure to hyperosmotic stress. ABA accumulates under hyperosmotic conditions and triggers the closure of guard cells (to prevent water loss) in

consonance with expression of ABA-specific stress responsive genes (Leung and Giraduat, 1998; Taylor *et al*., 2000; Wilkinson and Devis, 2002). Also, ABA independent signaling pathways are activated (Ingram and Bartels, 1996). Studies performed towards understanding the underlying mechanisms of ABA signaling resulted in the identification of ABA receptors and secondary messengers (Hirayama and Shinozaki, 2007). ABA-mediated regulation of gene expression involves *cis* ABA responsive (ABRE) and coupling (CE) elements, required for ABA responsiveness, which have been shown to be present in the promoters of stress responsive genes (Michel, 1993; Hobo *et al*., 1999; Gomes-Porras *et al*., 2007). Proteins (ABFs) that bind to ABRE elements containing a basic leucine zipper (bZIP) motif activate ABA stress responsive genes (Choi *et al*., 2000; Jakoby *et al*., 2002). ABRE motif is somewhat akin to the G-box that functions in regulating plant gene expression in response to light conditions, wounding and anaerobiosis. On the other hand, dehydration response element (DRE) and DRE binding factors involved in ABA-independent regulation of gene expression during osmotic stress (Agarwal *et al*., 2006; Shinozaki and Shinozaki, 2006; Abe *et al*., 1997, 2003), showed that dehydration response gene RD22BP1, and ATMYB2, MYC and MYB related proteins act as transcriptional activators of rd22 (dehydration response) in response to drought and ABA, respectively. Leung *et al*. (1994, 1997) isolated and characterized Arabidopsis ABI1 and ABI2 homologous (ABA insensitive 1 and 2) loci, which are crucial for plant responsiveness to ABA and negatively regulate ABA signaling (Goaniasti, 1999). ABI1 and ABI2 encode a serine-threonine protein phosphatase 2C (PP2C) (Bertauche *et al*., 1996). Thus, it has become clear that protein phosphorylation plays a key role in ABA signaling.

The list of protein kinases implicated in plant responses to osmotic stress is growing. For instance, highly conserved mitogen activated protein kinase (MAPK) cascade in osmotic signaling is well established in yeast, plants and mammals (Johnson and Lapadat, 2002; Zhang *et al*., 2006; Raymond and Thorner, 2007). MAPKs are also induced by drought, cold, wounding, hormones, and plant pathogen interactions (Jonak *et al*., 1996; Bogre *et al*., 1997; Zhang *et al*., 2006). Besides MAPK, four other protein kinase superfamilies modulated by the secondary messenger Ca^{2+} are involved in stress signaling: Ca^{2+} dependent protein kinase (CDPK), CRK (Calcium related protein kinase), CCaMK (calcium and calmodulin related protein kinase) and SnRK3 (Snf1 related kinase 3, classified as CDPK-SnRK) (Harper *et al*., 2004). The focus of this review is on plant SnRK2 (SNF1-Related Protein Kinases 2) family and their role in ABA and hyperosmotic signaling.

Snf1 Related Kinase (SnRK) Gene Family

Plant SnRKs have been grouped into three subfamilies: SnRK1, SnRK2 and SnRK3 (Hardie *et al.*, 1998; Hrabak *et al.*, 2003), where SnRK1 has a role in global regulation of carbon and nitrogen metabolism, and SnRK2 and SnRK3 in stress signaling. Interestingly, recent studies have revealed a direct connection between ABA signal transduction and SnRK2 family members. SnRK1 is the plant homolog of yeast (*Saccharomyces cerevisiae*) SNF1 gene that encodes a protein kinase essential for expression of glucose repressible genes in yeast (Celenza and Carlson, 1986). SnRK1 and SnRK2 family of protein kinases are similar in their catalytic domains but have divergent C-terminal domains while SnRK3 protein family forms a unique group.

Snf1 Related Kinase 1 (SnRK1)

SnRK1 stands for sucrose non-fermenting 1-related protein kinase-1 because of its homology and functional similarity with sucrose non-fermenting-1 SNF1 kinase in yeast. The first plant SnRK1 cDNA sequence was isolated from a rye endosperm cDNA library, encoding a 57.7-kDa protein with 502 amino acid residues (Alderson *et al*., 1991). SnRK1 shares 48 % amino acid sequence identity with yeast SNF1 and AMP kinases (AMPK) while the catalytic domains are 62-64 % identical. These kinases regulate energy metabolism (Barker *et al*., 1996, Halford *et al*., 2003), similar to the suggested roles of the mammalian AMPK and yeast SNF-1 (Hardie *et al.*, 1998; Hardie, 2007). In barley, SnRK1 is required for the formation of functional pollen, possibly because it is needed for starch accumulation (Zhang *et al.*, 2001). In potato, SnRK1 is necessary for sucrose-induced expression of sucrose synthase (Purcell *et al*., 1998). An embryo-expressed SnRK1 has been suggested to regulate the expression of α-amylase involved in starch accumulation in wheat (Laurie *et al*., 2003). A function for SnRK1 in metabolism homeostasis in the dark has also been proposed (Thelander *et al*., 2004).

Snf1 Related Kinase 2 (SnRK2s)

The first SNRK2 cDNA clone (PKABA1) was isolated from an ABA-treated wheat embryo cDNA library (Anderberg *et al*., 1992). Subsequently, another member of SnRK2 subfamily was identified as a central regulator of AAPK (ABA activated protein kinase) involved in ABA-dependent stomatal closure in fava bean (Li *et al*., 1996; 2000). OST1 (open stomata 1/SnRK2.6/OST1/

SRK2E), an Arabidopsis ortholog of AAPK, is required for the regulation of stomatal aperture by ABA (Mustilli *et al*., 2002). In soybean, expression of two cDNA clones, SPK3 and SPK4, increased in response to dehydration and high salinity, but with different induction kinetics (Yoon *et al*., 1997). SPK3 is induced by exogenously supplied ABA while SPK4 remains unaffected. Two other members of SnRK2s subfamily, SPK1 and SPK2, from soybean were shown to be activated by hyperosmotic stress (Monks *et al*., 2001). Kobayashi *et al*. (2004) identified 10 SnRk2 protein kinases from rice (*Oryza sativa*) genome and expressed them in cultured cell protoplasts. They demonstrated that all family members were activated by hyperosmotic stress but only three (SAPK 8, SAPK 9 and SAPK 10) by ABA. Similarly, ten members of Arabidopsis SnRK2 family were expressed and analyzed in cells and seedlings. Ten SnRK2s are encoded by *Arabidopsis* genome, of which SnRK2.2, SnRK2.3, SnRK2.6/OST1/SRK2E, SnRK2.7, and SnRK2.8/SnRK2C are activated by ABA (Boudsocq *et al*., 2004). At least four members of this family were found activated *in vivo* during hyperosmotic stress, while in a transient expression system nine of them were activated by hyperosmotic stress using mannitol and NaCl (Boudsocq *et al*., 2004). Based on the amino acid sequence, a 42-kDa protein kinase (*NtOSAK*) activated by osmotic stress in tobacco (*Nicotiana tabacum*) has been assigned to the SnRK2 family (Kelner *et al*., 2004). A maize SnRK2b homologue (ZmSPK1) was shown to be expressed in roots, mature leaves and vassels but most abundantly in the reproductive organs (Zou *et al*., 2006).

Dehydration, osmotic stress and ABA all induced an OSRK1 gene identical to SAPK6 (Kobayashi *et al*., 2004), encoding a 41.8-kDa protein kinase belonging to the SnRK2 family from *Oryza sativa*. This protein kinase showed a preference for an uncommon cofactor requirement for Mn^{2+} over Mg^{2+} (Chae *et al*., 2007). Using reverse genetics approach, Fujii *et al*. (2007) isolated single and double mutants of SnRK2.2 and SnRK2.3, and found that the double mutant was insensitive to ABA during seed germination and seedling growth indicating a role for SnRK2.2 and SnRK2.3 protein kinases in mediating ABA signaling during seed germination.

Snf1 Related Kinase 3 (SnRK3)

Like SnRK2, SnRK3 gene subfamily is also unique to plants (Halford *et al*., 2003) but comparatively more diverse and larger than SnRK1. Most studies on SnRK2 and SnRK3 kinases have focused on their involvement in different stresses. Several SnRK3 subfamily kinases have been studied, one of which is an *Arabidopsis* protein kinase involved in conferring salt tolerance, SOS2 (salt overly sensitive 2) (Halfter *et al*., 2000; Liu *et al*., 2000). *Arabidopsis* PKS3, PKS18 or CIPK3 members of SnRK3 family modulate ABA sensitivity in seedling growth, stomatal closure and seed germination (Gong *et al*., 2002; Guo *et al*., 2002; Kim *et al*., 2003). Another SnRK3 subfamily kinase is the wheat (*Triticum aestivum*) WPK4, whose expression is down regulated by sucrose and up regulated by light, cytokinins and low temperature (Sano and Youssefian, 1994; Ikeda *et al*., 1999).

Structural Analysis of SnRK2

SnRK2 kinases are about 140 to 160 amino acids shorter than SnRK1s, encoding a protein of about 40 kDa with a characteristic stretch of acidic amino acids, either poly-Glu or poly-Asp, at their C-terminal regions (Hardie, 1999; Halford *et al*., 2000). The catalytic domain of SnKR2 kinases shows 42-46% amino acid sequence identity with plant SnRK1, SNF1 and AMPK. However, SnRK2 and SnRK1 are completely divergent at the C-terminal regulatory domains and do not interact with the activating and substrate targeting subunit of SnRK1 (Hrabak *et al*., 2003). Two conserved boxes are located in the carboxy-terminal region, after the catalytic domain, of SnRK2.6/OST1/SRK2E, a member of SnRK2, the SnRK2-specific box (glutamine-303 to proline-318) and the ABA-specific box (leucine-333 to methionine-362) (Belin *et al*., 2006). OSRK1 represents another dehydration inducible gene (1522 bp in length, 1,095 bp of coding region, 94 bp of the 5'untranslated region, and 123 bp of 3'untranslated region) from rice (Chae *et al*., 2007).

SnRK2 Substrates and Interacting Proteins

Knowledge of the substrate(s) and interacting protein(s) provides a better understanding of the enzymatic function of a gene product. Specificity of substrate is not only helpful in monitoring the activity of enzymes but also in the search for endogenous target proteins. Several studies have reported the substrates phosphorylated by SnRK2 in an ABA specific manner in different plant species. AAPK-interacting protein 1 (AKIP1), a heterogeneous nuclear RNA binding protein as a substrate of AAPK, is involved in the ABA regulation of post-transcriptional RNA metabolism in *Vicia faba* (Li *et al*., 2002). bZIP transcription factors, such as TaAbF from wheat (Johnson *et al*., 2002) and TRAB1 from rice (Kagaya *et al*., 2002), were shown to be substrates for SnRK2. Prediction for a sequence motif for a substrate recognized by NtOSAK (Kelner *et al*., 2004) came about using the computer program PREDIKIN (Brinkworth *et al*., 2003). This motif, [KR]-[QMTAS]-X-[ST]-[VILMF]-

[SQN]-[FLIRK], has some similarity with a consensus sequence present in the substrates of SNF1/AMPK protein kinases (Dale *et al*., 1995). NtOSAK protein phosphorylates with nearly equal efficiency peptides SAMS and AMARA (designed as specific substrates for SNF1/AMPK protein kinase), GST-ACC (natural AMPK substrate, acetyl-CoA carboxylase), myelin basic protein (MBP), and casein (Kelnar *et al*., 2004).

Two hybrid yeast system identified a phosphatase that likely interacts with SRK2E/OST1/SnRK2.6. It was identified as *ABI1*, which encodes a PP2C-type phosphatase and negatively regulates many aspect of ABA action (Yoshida *et al*., 2006). ABI1 may bind OST1 and thereby modulate its phosphorylation status.

ABA responsive transcription factors, ABF/AREB, have been identified as SnRK2 substrates in rice (Kobayashi *et al*., 2005) and *Arabidopsis* (Furihata *et al*., 2006). By overexpression in Arabidopsis T87 cultured cells and use of an in-gel kinase assay, five ABA responsive AtSnRK2s (Furihata *et al*., 2006) and three rice SAPKs were shown to, respectively, phosphorylate AREB1b and TRAB1 (Kobayashi *et al*., 2005).

Dehydration inducible SnRK2 (OSRK1) is capable of autophosphorylation, and phosphorylates MBP and histone (Chae *et al*., 2007). OSRK1 can also phosphorylate OREB1, a rice homolog of ABI5/ABF/AREB subfamily of bZIP transcription factor, at multiple functional domains. A recognition motif for OSRK1 (R-Q-X-S/T) was established using peptide substrates. Using yeast two hybrid system, OSRK1 was shown to interact with OREB1 and co-localize to nucleus. An effective phosphoproteomics approach helped identify candidate target proteins phosphorylated by SnRK2.8: adenosine kinase, glyoxalase 1, ribose 5-phosphate isomerase (R5PI), ribosomal protein and three regulatory proteins in the 14-3-3 family (Shin *et al*., 2007).

Regulation and Activity of SnRK2

Reversible phosphorylation is used by living cells to regulate many cellular processes. In ABA-mediated signaling, protein kinase activation is a critical event, and it applies particularly to SnRK2 kinases in response to osmotic stress and ABA treatment.

In vitro treatment with phosphatases has shown that kinase phosphorylation is important for activation: Tobacco homolog of *Arabidopsis* SnRK2 (ASK1/SnRK2.4) purified from BY-2 cells was inactivated by protein phosphatase 2A (PP2A) (Miklajczyk *et al*., 2000; Kelner *et al*., 2004); alkaline phosphatase abolished salt activation of two rice SnRK2s, SAPK1 and SAPK2 (Kobayashi *et al*., 2004). Suppression of OST1 kinase activation in response to ABA in the dominant abi1-1 mutant (Mustilli *et al*., 2002) provided the evidence for negative regulation of ABA signal transduction upstream of OST1 by protein phosphatase 2C (PP2C) ABI1 (Koornneef *et al*., 1984; Leung *et al*., 1994). Autophosphorylation of bacteria-produced recombinant REK, SnRK2 from rice, was found to be dependent on Ca^{2+} ions, suggesting that calcium signaling may be involved in kinase function (Hotta *et al*., 1998).

In an incisive analysis, Boudsocq *et al*. (2007) demonstrated the phosphorylation mechanisms and at least three signaling pathways involved in the activation of SnRK1-2 by osmotic stress and ABA. As described above for other related kinases, phosphatase treatment abolished activation of SnRK2-3 and SnRK2-6 by osmotic stress as well as by ABA. Normally undetectable transcripts of dehydration-induced rice SnRK2 in vegetative tissues were found to surface upon hyperosmotic stress and ABA treatment (Chae *et al*., 2007). Burza *et al*. (2006) mapped the phosphorylation sites of NtOSAK by using NtOSAK immunoprecipitated protein from BY-2 cells and demonstrated that Ser-154 and Ser-158 residues are phosphorylated during osmotic stress. They also showed that phosphorylation of Ser-158 is essential for the activation of NtOSAK while Ser-154 facilitates the Ser-158 phosphorylation. These results are in accord with Belin *et al*. (2006) who produced recombinant OST1 after introducing point mutations in defined amino acid residues and constructed deletion mutants. They showed that phosphorylation of Ser-175 in OST1 kinase was essential for recombinant OST1 activity. Mutating Ser-158 and Thr-159 to aspartic acid in the activation loop of OSRK1 or deleting its C-terminal 73 amino acids significantly reduced the enzyme activity (Chae *et al*., 2007).

Monks *et al*. (2001) showed that when overexpressed in yeast, two soybean SnRK2s (SPK1 and SPK2) were activated by high hyperosmolarity. A *Vicia faba* AAPK homolog has been shown to mediate stomatal closure in guard cell protoplast, in an ABA dependent pathway (Li *et al*., 1996; 2000).

Interestingly, SnRK2.6/OST1/SRK2E is activated by drought and ABA while its transcripts' expression is not regulated by ABA. In another study, mutants of SnRK2.6 were shown to be affected in stomatal closure in response to ABA (Mustilli *et al*., 2002; Yoshida *et al*., 2002). Contrary to AtSnRk2.6, transcription of PKABA1 from wheat (*Triticum aestivum*), another member of SnRK2 family, is induced by ABA in embryos and seedlings (Gomez-Cadenas *et al*., 1999, 2001; Shen *et al*., 2001).

ABA-induced gene expression of two drought-inducible genes, *RD22* and *RD29B*, is suppressed in srk2e mutant (Yoshida *et al.*, 2002).

SRK2C, a SNF1-related protein kinase 2, is activated upon osmotic stress and improves drought tolerance of *Arabidopsis* plants (Umezawa *et al.*, 2004). SRK2C expression was tissue specific, being abundantly expressed in roots but weakly in leaves and siliques. These data suggested that SRK2C must function mainly in root tips. Overexpression of SRK2C was found to upregulate many stress-responsive genes, namely, RD29A, COR15A and DREB1A/CB3.

Arabidopsis thaliana SnRK2 (SnRK2.3) is similar to the sulfur-regulatory gene *SAC3* of *Chlamydomonas reinhardtii.* It was found that SnRK2.3 protein kinase is involved in regulating sulfur-responsive gene expression and accumulation of O-acetyl-L-serine under limited sulfur availability (Kimura *et al.* 2006).

CONCLUSIONS

ABA plays an important role in controlling many abiotic stress responses. Osmotic stress and ABA activate SnRK2 kinase by phosphorylation. Significant progress has been made in unraveling SnRK2s signaling. Identification of upstream kinases and interacting components involved in SnRK2 activation should help understand the SnRK2 transduction pathways in osmotic and ABA signaling. The tobacco NtOSAK, SnRK2 is able to phosphorylate SAMS and AMARA peptides, conserved substrates of SNF1/AMPK/SnRK1. This suggests that SnRK1 and SnRK2 possibly share common targets. Thus, the role of SnRK2 kinase family in cross-talk mechanism during stress signaling, interconnecting networks in plants will be an important area for future investigation. Several bZIP transcription factors have been identified as substrate of SnRK2 kinases.

We found OSRK1 (which phosphorylates OREB1) recognition motif in some *Arabidopsis* ABF/AREB/ABI5 bZIP transcription family members (Jacoby *et al.*, 2002). The rice TRAB1, which is phosphorylated by SnRK2, is the *Arabidopsis* ABI5 homolog. Nine out of thirteen ABF/AREB/ABI5 bZIP members contain the recognition motif, R-Q-X-S/T, as defined by the requirement for OSRK1 (Table 2). This analysis suggests that SnRK2 kinases can be major regulators of gene expression during hyperosmotic stress.

From a vast number of confirmed observations in the literature and the presence of SnRK2 recognition motif in bZIP transcription factors (Table 2), we synthesized a simple model of hyperosmotic and salinity stress-mediated signaling, depicted in Fig. 1. Hyperosmotic conditions lead to the release of ABA causing activation of SnRK2 kinases (SnRK2.6/OST1/SRK2E, PKABA1, SAPK8-10, OsRK1), which in turn phosphorylate members of bZIP/ABRE/AREB family of transcription factors. The phos relay system then switches on transcriptional and translational activities, leading to physiological changes in the subject plant. Based on the duration of this signaling, stress resistance or stress tolerance may be accomplished. Another group of SnRK2 kinases (SnRK2.1, SnRK4,5,10, SPK4, SAPK1-7,

Table 1. SnRK2 protein kinases and their known interacting partners

SnRK2 protein	Species	Interacting partner	Class	Method	Ref.
AAPK	*V. faba*	AKIP	RNA binding protein	Yeast two hybrid	Li *et al.*, 2002
PKABA1	*T. aestivum*	TaAbF	bZIP	Yeast two hybrid	Johnson *et al.*, 2002
OST1/SnRK2.6	Arabidopsis	ABI1	PP2C phosphatase	Yeast two hybrid	Yoshida *et al.*, 2006
SnRK2.8 SnRK2.2 SnRK2.6 SnRK2.7 SnRK2.3	Arabidopsis	AREB	ABRE transcription factor	In gel kinase assay	Furihata *et al.*, 2006
SAPK10	Rice	TRAB1	bZIP	Co-immuno-precipitation	Kobayashi *et al.*, 2005
OSRK1/SAPk6	Rice	OSRK1	bZIP	Yeast two hybrid	Chae *et al.*, 2007

Table 2. Members of ABF/AREB/ABI5 bZIP transcription family with a similar recognition motif (R-Q-X-S/T) in basic region shared with OSRK1 and rice TRAB1[H]

Locus	Protein	Amino acid residues
AB023288	TRAB1	82-85
NM_103859	ABF1	26-29, 93-96, 132-135
NM_179446	ABF2	23-26, 84-87, 132-135
NM_179159	ABF3/DPBF5	29-32, 123-126, 166-169
NM_112816	ABF4	36-39, 111-114, 152-155
NM_129185	ABI5	39-42, 142-145, 198-201
NM_115544	AREB3	18-21, 78-81
NM_201926	EEL	22-25,66-69
AJ419599	AtbZIP15	22-25, 66-69
NM_114314	DPBF2	37-40, 95-98, 140-143

[H]A similar motif was not found in: NM_100278, GBF4; NM_127331; AtbZIP27/FDP; ATH000023, AtbZIP13; ATH000021, AtbZIP14.

SAPK1,2) is activated by various osmolytes (NaCl, mannitol, sucrose). Their activation in response to salt is quick, which suggests that these, and others to be yet identified, salt activated SnRK2 kinases may be specifically involved in osmotic stress signaling (Boudsocq *et al*., 2004). Direct interaction of OST1/SRK2E/SnRK2.6 with ABI1 (PP2C) and inhibition of OST1/SRK2E/SnRK2.6 activity by ABA in abi1-1 mutant strongly suggest that plants switch on an intricate phosphorylation-dephosphorylation mechanism in response to ABA. Likewise, the kinase partners in salinity-induced kinase signaling, SAPK1 and SAPK2, are affected by a phosphatase (alkaline) as well (Fig. 1). In *Vicia faba*, AAPK interacts with AKIP1, an RNA binding protein, suggesting that SnRK2 may also be recruited for post-transcriptional regulation.

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This article is dedicated to honor Prof. Sudhir K. Sopory on his 60th birthday. Prof. Sopory has immensely contributed to various aspects of plant biology and a major part of his career has involved research towards understanding signaling mechanisms in plants with particular emphasis on abiotic stresses. In 1980's, Prof. Sopory spent a 14 month sabbatical in Mattoo laboratory

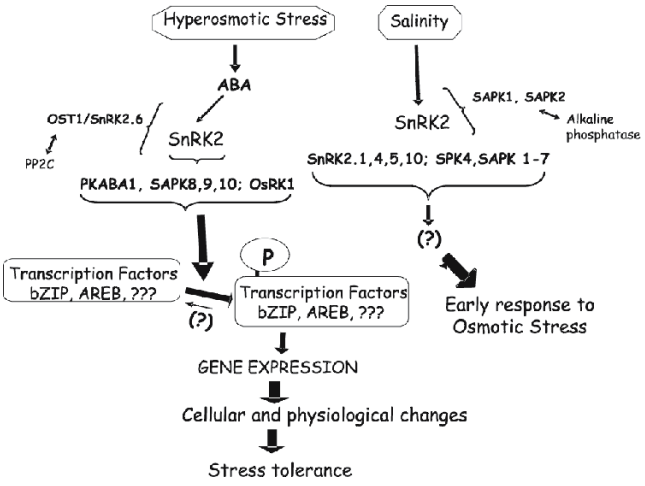

Fig. 1. A scheme illustrating components, sequence of signaling networks and regulation of SnRK2 kinases by hyperosmotic stress and salinity. Osmotic stress causes SnRK2 kinase activation via the plant osmotic response hormone, ABA. In turn, bZIP transcription factors get phosphorylated, causing a series of transcriptional and translational changes that may lead to stress tolerance of a plant. Salinity induces SnRK2 independent of ABA and such a quick response likely primes the tissue for achieving metabolic homeostasis in response to high salinity conditions. ABA also activates OST1/SnRK2.6 that interacts with the PP2C phosphatase while salt-activated SAPK1 and SAPK2 are known to interact with alkaline phosphatase, suggesting that dephosphorylation of protein kinases is another key process during osmotic stress.

at Beltsville, Maryland. His contributions with Mattoo group in the field of photosystem II (PSII) and photosynthesis include: the first demonstration that reactive oxygen species (ROS) may be a factor in the light-dependent degradation of the D1 PSII reaction center protein (Sopory *et al.*, 1990), the first demonstration of a transiently modified, phosphorylated form of the D1 PSII reaction center protein (Callahan *et al.*, 1990), and the first demonstration of the presence of a PSII reaction center complex in the stromal lamellae (Ghirardi *et al.*, 1993). These findings were seminal in

charting the life of this very important protein in photosynthesis (Sopory *et al.*, 1993), and were recently recollected (Edelman and Mattoo, 2006).

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