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The Orthologues of ABA Receptors and ABA Signaling Components in Rice

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ABSTRACT

Abscisic acid (ABA) is a multi-functional plant hormone that acts in several different physiological processes such as stomata closing, seed dormancy, abiotic stress adaptation and developmental differentiation. Many efforts have been made over the last decades to identify the molecular mechanisms of ABA signal transduction pathways. In particular, the identification of the ABA receptors has been one of the most important issues facing this research area. Recently, ABA receptors, including two GPCR-type G proteins, a Mg-chelatase H subunit and PYL/RCARs were reported to bind ABA and to be involved in ABA-dependent responses in seed dormancy, stomata closure and abiotic stress adaptation in *Arabidopsis thaliana*. In particular cytosolic ABA receptor PYL/RCARs are considered the major regulators of ABA dependent gene expression. The signaling components consisted of PYR/RCAR, subclass A PP2C, SnRK2 and ABF studied well and the crystal structures of the components and complexes were identified in Arabidopsis. In this review, we describe ABA receptors and signaling components of Arabidopsis and identify the rice orthologues corresponding to ABA receptors and signaling components of Arabidopsis by homology searches in the rice database. This also suggested that the receptors and signaling components of ABA are highly conserved in dicot and monocot plants evolutionarily.

Keywords: ABA receptor, PP2C, SnRK2, rice, signal transduction
Abbreviations: ABA, abscisic acid; **CDS**, coding sequence

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INTRODUCTION

In order to survive in adverse environments, plants have evolved intricate systems to recognize and respond to changes in the environment. Plants have a sessile life style and need to be able to survive under unfavorable conditions. Thus, tolerance systems for environmental stresses are indispensable. In plants, one of the mechanisms for overcoming abiotic stresses involve synthesis of abscisic acid (ABA), thereby increasing the ABA concentration and leading to expression of stress-responsive genes through a signal transduction pathway that recognizes and responds to ABA in the cell (Leung and Giraudat 1998; Wasilewska *et al*. 2008).

In the 1960's, ABA was first isolated from cotton as a chemical named abscisin II that promoted the abscission of cotton fruits (Ohkuma *et al.* 1963). Abscisin II was found to be the same chemical with the dormin known as a growth inhibiting compound isolated from sycamore (Cornforth *et al*. 1966), and those were later renamed ABA (Addicott *et al*. 1968). After that, ABA was discovered in all vascular plants and mosses except for liverworts, and this ubiquitous hormone is now known to be synthesized in plant chloroplasts via the carotenoid pathway.

ABA is a multi-functional plant hormone that plays a key role in several different physiological processes, including seed dormancy, abiotic stress tolerance, senescence, and developmental differentiation, and mediates stress responses, including stress-responsive gene expression, stomata closure and vegetative growth modulation (Adie *et al*. 2007; McCourt and Creelman 2008; Rodriguez-Gacio Mdel *et al*. 2009). Several components of ABA signaling have been identified and characterized, including both positive and negative regulators (Wasilewska *et al*. 2008). However, for a long period, the ABA receptor was not known despite the great deal of effort focused on identifying it (McCourt and Creelman 2008).

Several lines of evidences suggested that ABA receptors are localized on the plasma membrane or in the cytosol. In recent years, independent research groups have reported

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two different types of ABA receptor candidates. GPCR-type G proteins, GTG1 and GTG2, were reported as putative membrane-localized ABA receptors (Pandey *et al*. 2009). GTG1 and GTG2 bind ABA specifically and interact with the G protein α subunit. In addition, mutants lacking GTG1 and GTG2 exhibit ABA hypersensitivity (Pandey *et al*. 2009). CHLH, a plastid-localized subunit of the Magnesium protoporphyrin-IX chelatase (Mg^{2+} chelatase), was also identified as a putative ABA receptor. It was first identified from broad bean as an ABA-binding protein. In Arabidopsis, CHLH specifically binds ABA and mediates ABA signaling as a positive regulator in seed germination, post-germination growth and stomata movement (Shen *et al*. 2006). Flowering time control protein A (FCA), an RNA-binding protein that regulates flowering time in Arabidopsis was also reported to function as an ABA receptor. However, the binding properties of FCA to ABA were questioned, and the original article claiming that FCA is an ABA receptor was retracted (Razem *et al*. 2006; Risk *et al*. 2008).

In 2009, two independent research groups reported that cytosolic ABA receptors, PYR/RCARs mainly regulate ABA-dependent gene expression signaling (Ma *et al*. 2009; Park *et al*. 2009). These proteins bind ABA and interact with sub-group A protein phosphatase 2Cs including ABI1 and ABI2. Thus, a complete signaling pathway consisting of ABA receptor, PP2C, SNF-1 related serine/threonine-protein kinase 2 (SnRK2) and ABRE binding factors (ABF) has been identified (Kuhn *et al*. 2006; Nakashima *et al*. 2009; Sirichandra *et al*. 2010).

Although the ABA receptors and ABA signaling components are important targets to improve the abiotic stress tolerance in crops, the ABA receptors were not studied yet except for the recent publication for OsPYL/RCARs of rice in monocot plants including most of crops (Kim *et al*. 2012). Thus, we will describe ABA receptors and signaling components of Arabidopsis and identify the rice orthologues corresponding to ABA receptors and signaling components of Arabidopsis by homology searches in the rice database. The information might be helpful and valuable for the researchers to study the ABA signaling or to improve the abiotic stress tolerance of crops.

ABA RECEPTORS IN RICE

The rice orthologues of ABA receptors, atGTG1 and atGTG2 localized on the plasma membrane

Several different studies showed that ABA receptors could be localized either on the plasma membrane or in the cytosol. ABA could bind to intact guard cell protoplasts of *Vicia faba* and this binding was eliminated by trypsin treatment (Weiler 1984). It was evidence that ABA receptors are present on the plasma membrane. On the other hand, direct injection of ABA into the cytoplasm of *Commelina communis* guard cells promoted stomata closure (Schwartz *et al*. 1994). It shows that ABA receptors are localized in the cytoplasm. Consistent with these results, different types of ABA receptors have recently been identified on the plasma membrane and in the cytoplasm.

G-protein coupled receptors (GPCRs) have been considered to be ABA receptor candidate localized on the plasma membrane for a long time. Although, plants have very few G-protein signaling components, plants also have heterotrimeric G-proteins composed of G_α, G_β, and G_γ subunits. These G-proteins transduce signals through interaction with G-protein coupled receptors (GPCRs) (Pandey *et al*. 2006). GCR1, a member of the GPCR family, was reported to mediate ABA signaling and interacted with G protein α-subunit GPA1 (Pandey and Assmann 2004). A *gcr1* T-DNA mutant showed ABA-hypersensitive phenotype in root growth, gene expression and stomata response. However, GCR1 itself is not the ABA receptor because GCR1 does not show ABA binding activity. After all, these findings demonstrated that G-protein signaling is involved in ABA signaling in plants (Pandey and Assmann 2004; Pandey *et al*. 2006).

A further study on GPCRs reported that GCR2 was a plasma membrane receptor for ABA (Liu *et al*. 2007). However, another research group has reported that GCR2 is unlikely to play a role as an ABA receptor in seed germination or early seedling development and also that its conformation is not typical of GPCRs (Gao *et al*. 2007). Thus GCR2 does not seem to be considered an ABA receptor at present.

However, other GPCRs have also been reported to be plasma membrane-localized ABA receptors. GTG1 and GTG2 identified from Arabidopsis fulfilled the criteria for an ABA receptor localized on the plasma membrane. GTG1 and GTG2 are plasma membrane-localized proteins having receptor-like topology and show specific ABA binding activity. The ABA-hyposensitive phenotypes of the *gtg1gtg2* double mutant and the dependency of ABA binding efficiency on their conformations support the conclusion that GTG1 and GTG2 are plasma membrane-localized ABA receptors (Pandey *et al*. 2009).

BLAST analysis of atGTG1 (At1g64990) and atGTG2 (At4g27630) using the rice protein database identified only one close homolog in rice. The rice GTG1 orthologue has two different splicing variants, The CDS of Os04g51180.1 consists of 1407 nucleotides encoding 469 amino acids named OsGTG1.1, and the CDS of Os04g51180.2 consists of 1044 nucleotides encoding 348 amino acids named OsGTG1.2. The amino acid sequence of OsGTG1.2 is shorter than OsGTG1.1 in C-terminal region. Arabidopsis GTG1 and GTG2 show very high amino acid sequence identity to each other, and the rice GTG protein OsGTG1.1 have 80% and 82% identity at the amino acid level to Arabidopsis atGTG1 and atGTG2, respectively (**Fig. 1A**). OsGTG1.1 also shares sequence similarity with human GPR89 (~45% identity and $8e^{-12}$ E-value). To investigate the topology of the plasma membrane-localized ABA receptor, we examined the predicted transmembrane domains of atGTG1, OsGTG1.1, OsGTG1.2 and HsGPR89 in the transmembrane prediction server (http://www.sbc.su.se/~miklos/DAS/maindas.html). The results showed that OsGTG1.1 has a similar nine predicted transmembrane domains with number of those of GTG1, GTG2 and GPR83, but OsGTG1.2 has quite different number of predicted transmembrane domains because of the short polypeptide compared to OsGTG1.1. Prosite motif analysis (http://www.expasy.ch/prosite/) identified a conserved ATP-/GTP-binding region in only OsGTG1.1 but not in OsGTG1.2. Taken together, OsGTG1.1 is likely to be the rice ABA receptor orthologue corresponding to Arabidopsis GTG1 and GTG2 but not OsGTG1.2 (**Fig. 1B**).

The rice orthologues of an ABA receptor, CHLH localized on the chloroplast

Biochemical approaches to identify the ABA receptor have been focused on isolating ABA-binding proteins. Zhang *et al*. (2002) reported the purification of a 42 kilodalton ABA-specific binding protein from the epidermis of broad bean leaves (Zhang *et al*. 2002). The same research group identified the protein and isolated the complementary DNA fragment based on the sequencing information. This cDNA encoded the carboxy-terminal half (~770 amino acids) of the putative H subunit (CHLH) of the magnesium protoporphyrin-IX chelatase (Mg-chelatase) (Shen et al. 2006). However, there is a controversy regarding whether it is a real ABA receptor. Tsuzuki *et al*. (2011) reported that Mg-chelatase H subunit could not bind ABA using ^{3}H-labelled ABA and a missense mutant of CHLH showed a phenotype in which stomata movements were insensitive to ABA although the plants displayed normal sensitivity to ABA with respect to seed germination and root growth (Tsuzuki *et al*. 2011). The Zhang group subsequently reported several additional lines of evidence that CHLH is a true ABA receptor. They found that the binding site of ABA in CHLH is located in C-terminal region and that mutants altered in the N-terminal region showed ABA-related phenotypes in

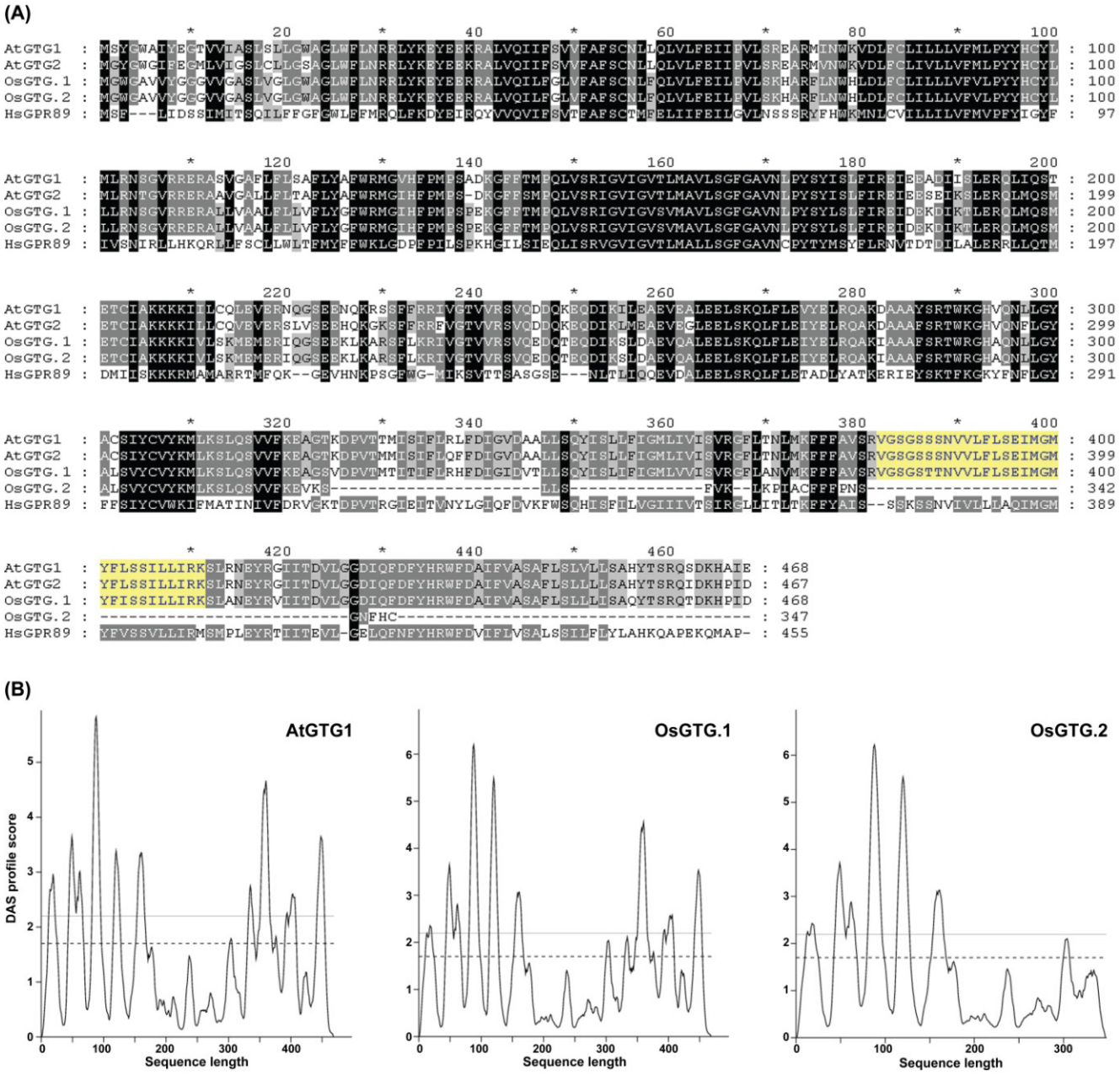

Fig. 1 Amino acid sequences alignment and transmembrane region prediction of GTG proteins. (**A**) Sequence alignment of the GTG proteins of rice and Arabidopsis with the human GPR89 protein. The alignment was performed with two AtGTG proteins; two OsGTG proteins derived from different splicing forms of one gene and human GPR89 protein using clustal W and GeneDoc program. Yellow marked amino acids represent the ATP-GTP biding site predicted by PROSITE. (**B**) Predicted transmembrane regions of GTG proteins. Transmembrane regions were predicted using "DAS" - Transmembrane Prediction server (http://www.sbc.su.se/~miklos/DAS/maindas.html). Solid line represents strict cutoff (2.2) and broken line represents loose cutoff (1.7).

seed germination and post-germination growth but not in stomata movement. Finally Mg-chelatase H subunit binds the WRKY transcription factor in an ABA-dependent manner and relieves the inhibition of ABA-responsive gene expression. Thus Mg-chelatase H subunit located on the envelope of chloroplasts seems to be a real ABA receptor (Wu *et al*. 2009; Shang *et al*. 2010).

Two rice homologues of Arabidopsis CHLH were found via BLAST analysis of the Arabidopsis Mg-chelatase H subunit (At5g13630) using the rice protein database. Mg-chelatase H subunit of Arabidopsis showed very high identity (82%) to rice CHLH1 (Os03g20700) at the amino acid level. OsCHLH1 has two different splicing forms, which are different only in the 3′ UTR region and have same amino acid sequence. The CDS of OsCHLH1 consists of 4164 nucleotides encoding 1388 amino acids. Another orthologue OsCHLH2 (Os07g46310) is shorter than OsCHLH1 due to the deletion of N-terminal region. It also showed high density (81%) and CDS of OsCHLH2 consists of 2283 nucleotides encoding 761 amino acids (**Fig. 2**). We analyzed prediction of chloroplast localization of the two rice CHLH orthologues using target P server (http://www.cbs.dtu.dk/services/TargetP/). OsCHLH1 was predicted to localize in chloroplast at high prediction value (0.671). This value was similar with AtCHLH (0.841). However OsCHLH2 was not predicted to be localized in chloroplast. Taken together, OsCHLH1 is likely to be the rice ABA receptor orthologue of Arabidopsis CHLH.

The rice orthologues of cytosolic ABA receptors PYL/RCARs

In 2009, the Cutler group of the University of California at Riverside reported to discover cytosolic ABA receptors using a chemical genomics approach (Park *et al*. 2009). They discovered pyrabactin, an analog of ABA, and isolated *pyrabactin resistant mutant 1* (*pyr1*), identifying the *PYR* gene using map-based cloning techniques. Fourteen

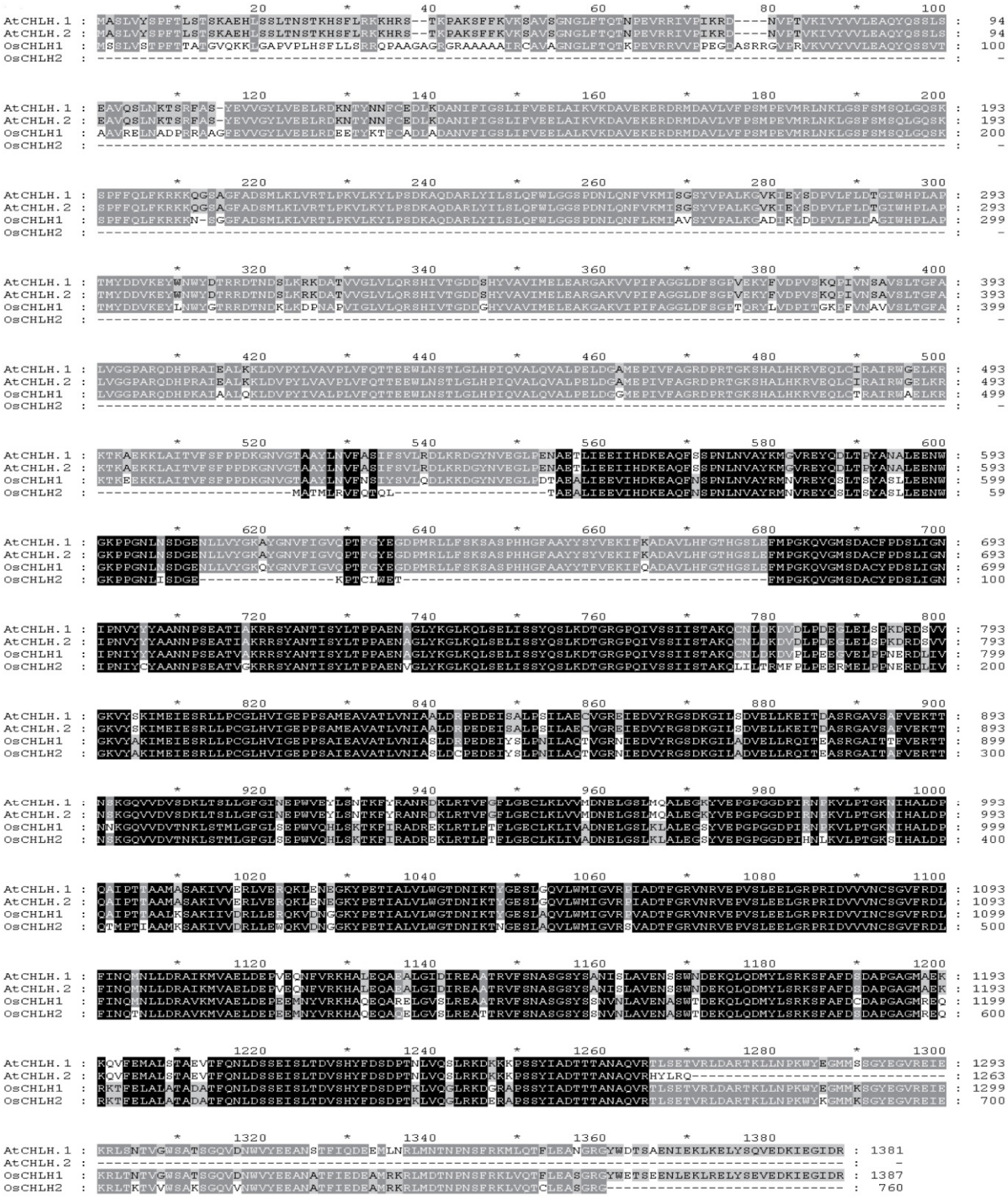

Fig. 2 Amino acid sequences alignment CHLH proteins. Sequence alignment of two different splicing forms AtCHLH and two rice CHLH like proteins. The residues that are completely conserved in both Arabidopsis and rice were highlighted in black using the GeneDoc program.

members of PYR and PYR Like protein (PYR/PYL) family are a subgroup of Bet v1 of birch pollen superfamily that contain a steroidogenic acute regulatory-related lipid transfer (START) domain. PYR/PYLs are able to bind ABA and exhibit ABA-dependent physical interactions with subclass A PP2CAs both in yeast and plants (Park *et al*. 2009).

Independently, another group also isolated ABA receptors by identifying the interactors of ABI1, a subclass A protein phosphatase 2C, using yeast two-hybrid screening. This interactor was referred to as RCAR (regulatory component of ABA receptors) (Ma *et al*. 2009). These cytosolic and soluble ABA receptors are now denoted as PYL/RCAR.

These cytosolic ABA receptors PYL/RCARs seem to be major positive regulator of ABA dependent gene expression. Once ABA bound PYL/RCARs interact with subclass A PP2Cs, PP2Cs are unable to dephosphorylate SnRK2 subclass III proteins. Phosphorylated SnRK2s are in an active state and able to phosphorylate the ABF transcription factors that bind ABRE elements on promoters. This leads to induction of ABA-responsive genes such as RD29A and RAB16 and, thus, the ABA signal alters the transcriptional patterns in plant cells (Fujii *et al*. 2009).

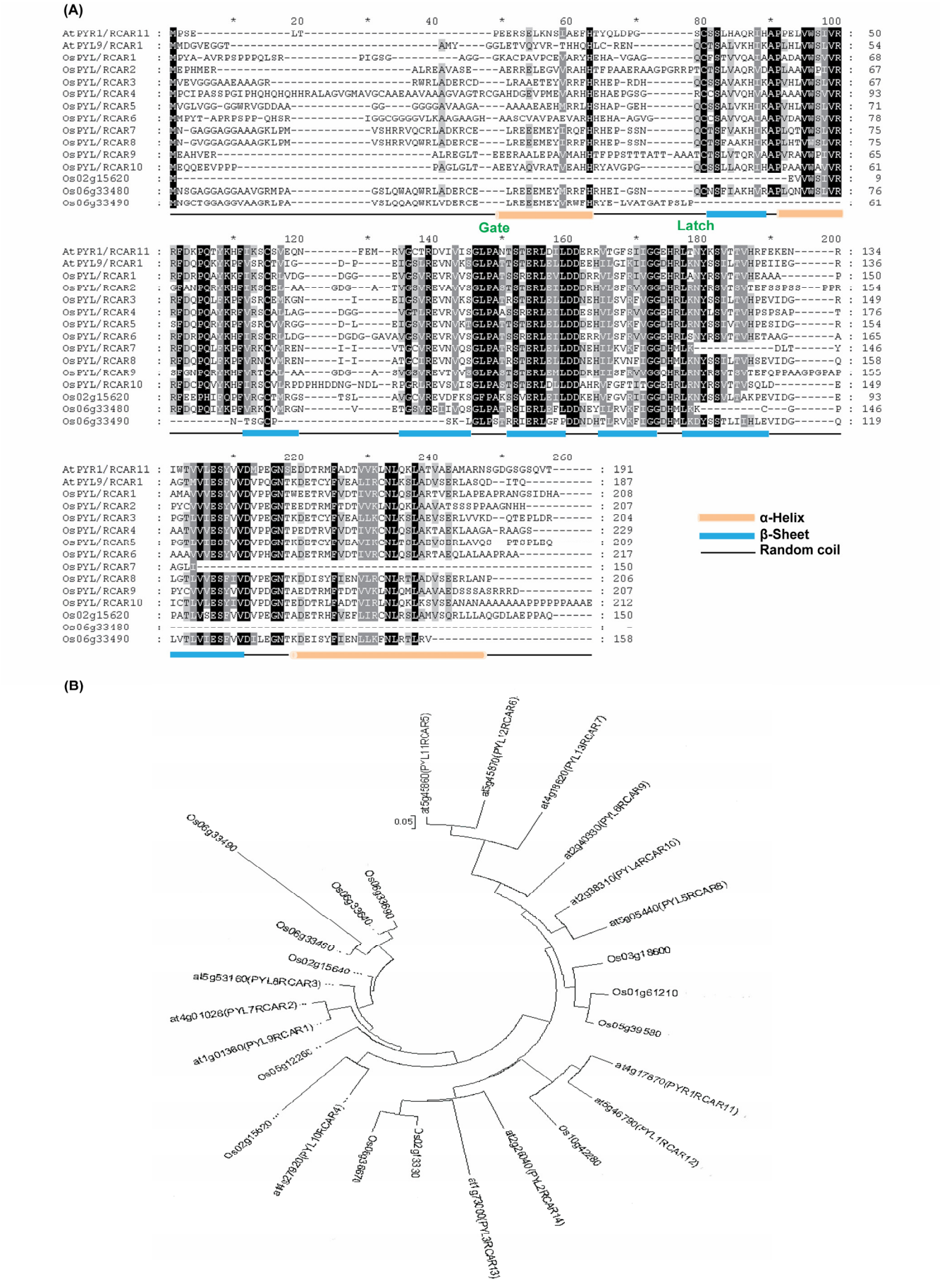

Fig. 3 Amino acid sequence alignment and phylogenetic analysis of PYL/RCAR proteins. (**A**) Alignment of two AtPYR/RCARs and 13 OsPYR/RCARs amino acid sequences. The residues that are completely conserved in both Arabidopsis and rice were highlighted in black using the GeneDoc program. Secondary structure was predicted by YASPIN program (http://www.ibi.vu.nl/programs/yaspinwww/). The amino acids consisting of structure of gate and latch were marked. (**B**) Phylogenic tree of rice orthologues and Arabidopsis PYL/RCARs was constructed by the ClustalW method using MEGA 5 program.

The components of this signal transduction pathway were reconstituted in Arabidopsis protoplasts and the reporter construct, in which luciferase was fused to ABRE elements, exhibited ABA-dependent transcription. This result shows that PYL/RCARs are positive regulators upstream of PP2C in ABA signal transduction (Fujii *et al*. 2009). After genetic and biochemical identification of PYL/RCAR as the cellular ABA receptor, several groups determined the protein structure of the PYL/RCAR and PP2C complex by X-ray crystallography. They showed that ABA binding changes the protein structure of the ABA receptors to expose the inter-phase where PP2C binds and that the interaction of PP2Cs with the ABA receptors locked the ABA binding pocket of the ABA receptors (Melcher *et al*. 2009; Nishimura *et al*. 2009; Peterson *et al*. 2010). The signal transduction pathway mediated by PYL/RCAR not only regulates ABA-dependent gene expression but also stomata closing by modulation of anion channel SLAC1 and KAT1 activity (Geiger *et al*. 2009; Lee *et al*. 2009; Sato *et al*. 2009). Two independent research groups reported that the PP2Cs and SnRK2s interact with SLAC1 antagonistically and regulate SLAC1 activity through phosphorylation/dephosphorylation (Geiger *et al*. 2009; Lee *et al*. 2009). This suggests that PYL/RCARs are the central sensors for transducing the ABA signal for stomata closing through SLAC1 and KAT1 regulation.

Functional rice orthologues of PYL/RCARs were reported by Kim *et al.* They isolated the homologue of PYR1 and overexpressed the gene in rice using maize ubiquitine promoter. Those transgenic plants showed the ABA hypersensitive phenotype in germination and post-germination growth and the ABA signaling components consisted of PYL/RCAR-PP2C-SnRK2-ABF are conserved and work as signaling unit for ABA dependent gene expression in rice (Kim *et al*. 2012).

In order to identify the rice orthologues of PYL/RCARs, we performed blast analysis using amino acid sequences of PYLs as queries and rice protein database at the Rice Genome Annotation site (http://rice.plantbiology.msu.edu/blast.shtml). Thirteen ABA receptor candidates that showed e-values lower than e^{-10} in BLASTP analysis were isolated. These genes were aligned by Clustal W and the amino acid residue identity between *Oryza sativa* and *Arabidopsis thaliana* was compared. In particular, Os02g15620 and Os06g33490 have deletions in the N-terminal region, which is well conserved in Arabidopsis and rice, and Os06g33480 has a deletion in the C-terminal region, which is also well conserved in other receptors. It was supposed that those genes might not be functional ABA receptors. Thus, it seems that there are 10 functional PYL/RCAR ABA receptors in rice (**Fig. 3**).

THE CORE COMPONENTS OF ABA SIGNALING: SUBCLASS A PP2C AND SNRK2

Although GPCR type G-proteins and chloroplast Mg-chelatase H subunit were reported to bind ABA and to be involved in seed dormancy and stomata closure, these receptors do not seem to regulate directly ABA-dependent gene expression mediated by subclass A protein phosphatase 2C (PP2C), SNF1-related serine/threonine protein kinase subclass 2 (SnRK2) and ABRE binding factors (ABF), which are the main ABA signaling components (Pandey *et al.* 2009; Shang *et al*. 2010). Discovery of cytosolic ABA receptor PYL/RCAR completed the ABA-dependent gene expression signaling pathway consisted of PYR-PP2C-SnRK2-ABF (Park *et al*. 2009). After the completion of ABA signaling networks by genetic, biochemical and cell biological approaches, many research groups identified the protein structures of each components and complexes to understand the molecular functional mechanisms (Melcher *et al.* 2009; Nishimura *et al.* 2009; Peterson *et al.* 2010; Soon *et al*. 2012). We are describing the advances in these research areas and compared the rice ortholgous of subclass A PP2Cs and SnRK2s with those of Arabidopsis in this section.

Rice orthologues of subclass A PP2Cs

Firstly, pharmaceutical approaches showed that intricate kinase and phosphatase networks are involved in ABA dependent gene expression and signaling. K-252a, a broad range protein kinase antagonist inhibited ABA dependent gene expression in pea epidermal peels (Schmidt *et al*. 1995; Allen *et al*. 1999). Reversely okadaic acid, an inhibitor of PP1 and PP2A induced or inhibited ABA dependent gene expression depending on the plant species and tissues (Schmidt *et al*. 1995; Allen *et al*. 1999). These results implied that protein kinases and protein phosphatases PP1s and PP2As might play as positive and negative regulators respectively in ABA signaling.

On the other hands, genetic approaches led to identify serine/threonine phosphatase 2C (PP2C) as important components of ABA signaling. A number of Arabidopsis mutants showing the insensitivity to ABA were isolated and named as *abi* (ABA-insensitive mutant), *abi1*, *abi2*, *abi3*, *abi4*, *abi5* and so on (Leung *et al*. 1997; Rodriguez *et al*. 1998). ABI1 and ABI2 were found out to be negative regulators of ABA signaling. These are serine/threonine PP2Cs and belong to the subclass A of PP2C. Later, HAB1 and AtPP2CA were also reported to be the negative regulator of ABA signaling and belong to subclass A of PP2C. Thus, subclass A PP2Cs were known to be the major negative regulators of ABA signaling pathways (Saez *et al*. 2004; Yoshida *et al.* 2006).

In plant serine/threonine phosphatases can be divided into two groups, PP1 and PP2, based on their substrate specificity and pharmacological properties. Nine PP1 phosphatases have been identified in Arabidopsis. Based on their requirement of divalent cations for catalysis, PP2 phosphatases can be subdivided into three classes. PP2A phosphatases do not require divalent cations, whereas PP2B phosphatases require Ca^{2+}, and PP2C phosphatases require Mg^{2+} or Mn^{2+} and are not sensitive to the inhibitor okadaic acid (Rodriguez 1998; Xue *et al*. 2008; Singh *et al*. 2010).

The PP2C family including *ABI1* and *ABI2* is the largest group among phosphatases of plants. After the completion of genome sequencing in Arabidopsis and rice, The database analysis revealed that the PP2C family consisted of 76 and 78 members in Arabidopsis and rice, respectively, even though only six PP2Cs are found in the yeast (*Saccharomyces cerevisiae*) genome and 13 PP2Cs are present in mammalian cells (Cheng *et al*. 2000; Saito *et al*. 2008; Xue *et al*. 2008). It has been proposed that the PP2Cs might be involved in a number of cellular processes in plants (Schweighofer *et al*. 2004; Xue *et al*. 2008). Seventy-eight *PP2C* genes of rice were grouped into 11 subfamilies (A to K) according to Xue *et al*. (2008). Later, 90 OsPP2Cs family members were identified by an exhaustive genome-wide analysis of phosphatases, which also were similarly grouped into 11 subfamilies (from A to K) (Singh *et al*. 2010). Subclass A OsPP2Cs consists of 10 members including orthologuous of *ABI1*, *ABI2 HAB1* and *AtPP2CA* of Arabidopsis. These OsPP2Cs showed high identities with Arabidopsis subclass A PP2Cs and especially PP2C domain regions were highly conserved. However, N-terminal regions were variable and this region might be attributed specificity to the PP2Cs (**Fig. 4A**). Based on the phylogenic tree, these subclass A OsPP2Cs can be classified into three groups such as ABI1 and ABI2 group, AHG3 group and AHG1 group (**Fig. 4B**).

Rice orthologues of SnRK2

The firstly reported SnRK2 gene was isolated from wheat and named PKABA1 (protein kinase ABA1). PKABA1 was identified to be induced by ABA and dehydration and then PKABA1 was reported to act as a key factor in the suppression of GA-inducible gene expression in the aleurone layers of barley. Another SnRK2-type protein kinase AAPK

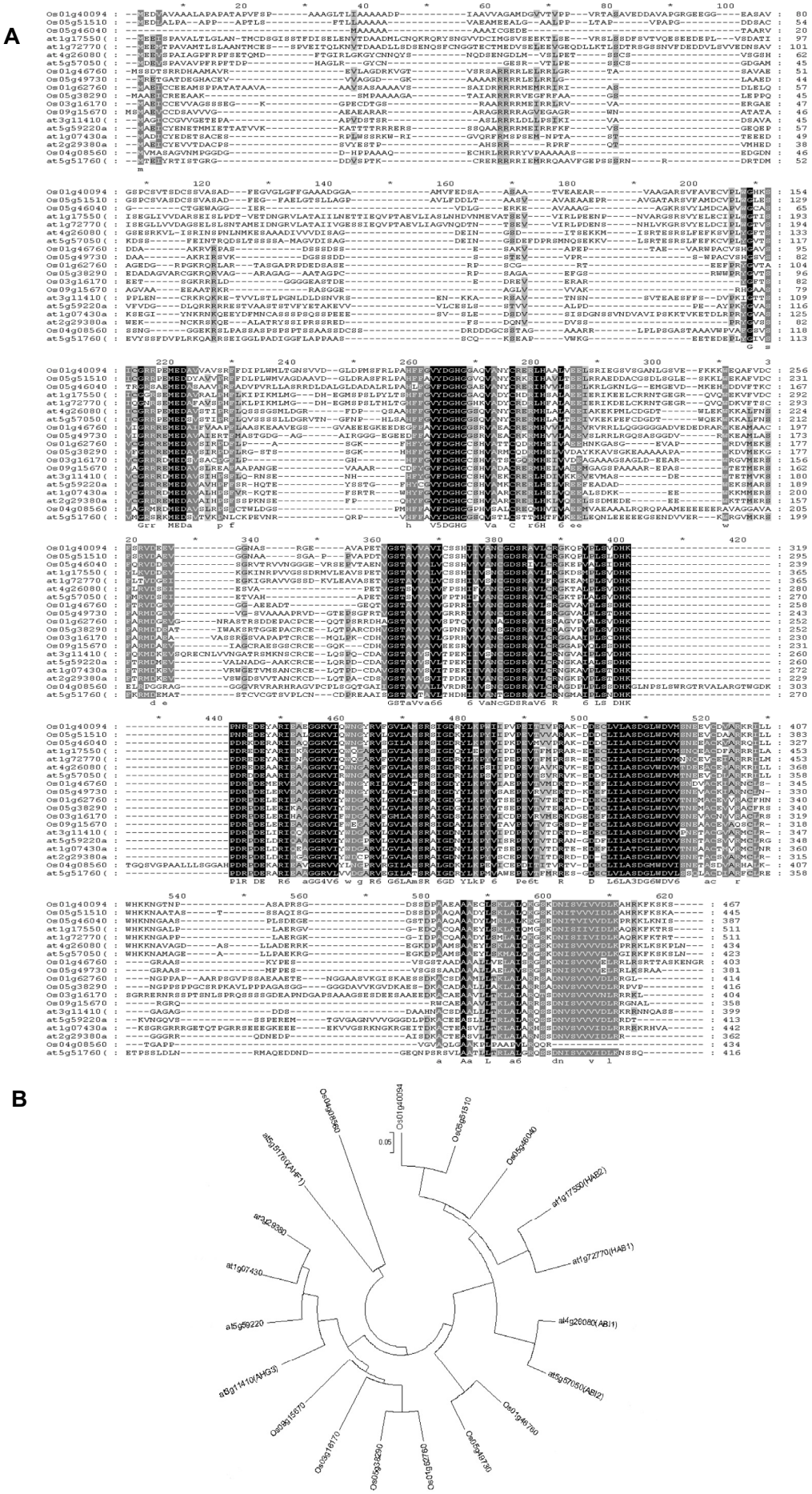

Fig. 4 Amino acid sequences alignment and phylogenetic analysis of subclass A PP2Cs. (**A**) Alignment of nine Arabidopsis subclass A PP2Cs and 10 rice orthologues amino acid sequences. The residues that are completely conserved in both Arabidopsis and rice were highlighted in black using the GeneDoc program. (**B**) Phylogenic tree of rice orthologues and Arabidopsis subclass A PP2Cs was constructed by the ClustalW method using MEGA 5 program.

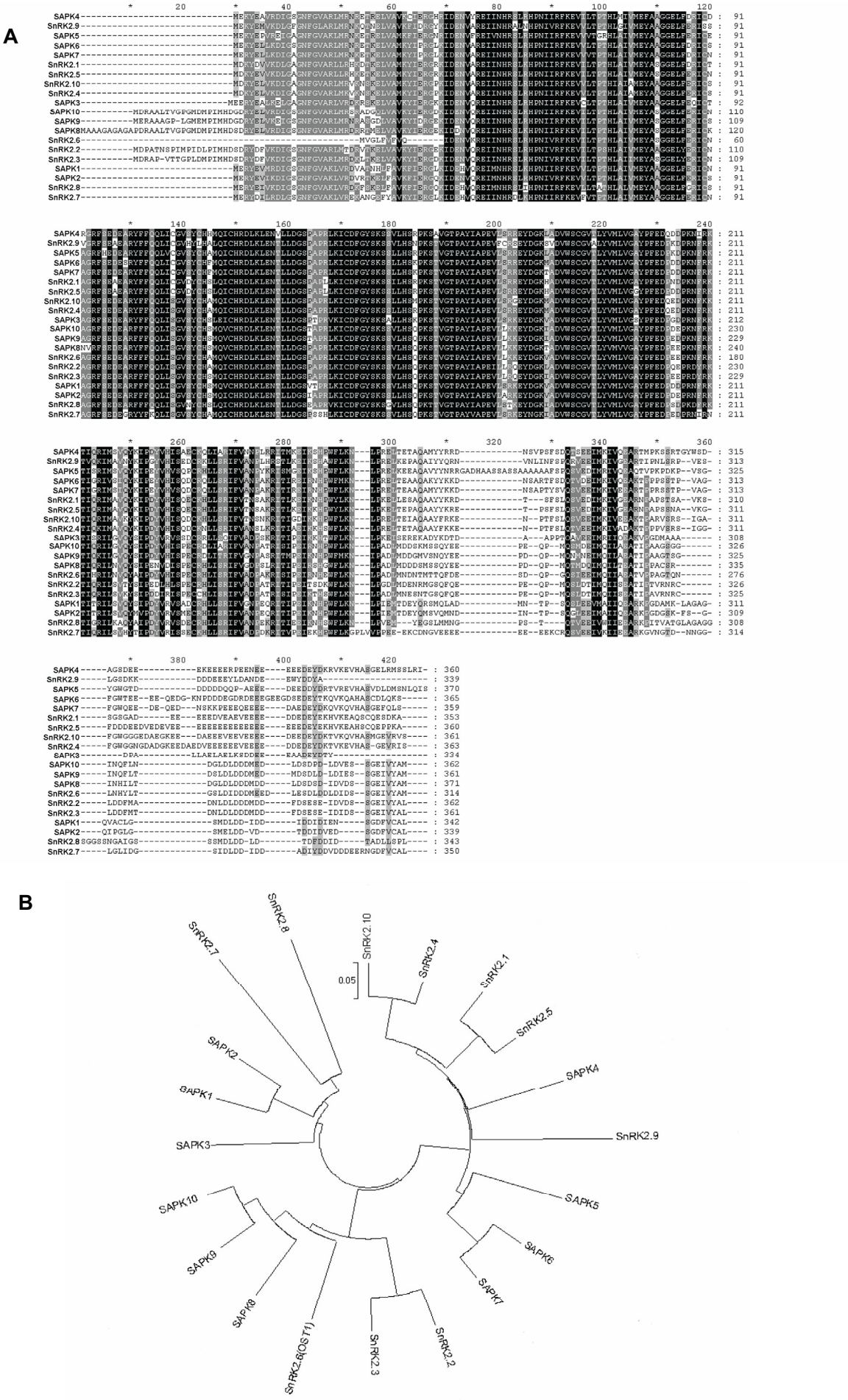

Fig. 5 Amino acid sequences alignment and phylogenetic analysis of SnRK2. (**A**) Alignment of ten Arabidopsis SnRK2s and ten rice SAPK amino acid sequences. The residues that are completely conserved in both Arabidopsis and rice were highlighted in black using the GeneDoc program. (**B**) Phylogenic tree of rice SAPKs and Arabidopsis SnRKs was constructed by the ClustalW method using MEGA 5 program.

was reported to be involved in the regulation of stomatal closure and activated in guard cell of *Vicia faba* (Anderberg and Walker-Simmons 1992; Gomez-Cadenas *et al*. 1999).

In Arabidopsis 10 SnRK2 members are designated SnRK2.1 through SnRK2.10 and classified into three subclass based on a phylogenetic analysis (Boudsocq and Lauriere 2005) (**Fig. 1**). Although SnRK2 was identified as an ABA activated protein kinase, hyperosmotic stress also activated the SnRK2s. Each subclass of SnRK2s have different activation patterns in relation to ABA and osmotic stress.

Subclass I SnRK2 kinases were not activated by ABA but rapidly activated by osmotic stresses. Subclass II kinase was very weakly activated by ABA in gel kinase assay. Subcalss III SnRK2s are strongly activated by ABA. Recently, triple knockout mutant of SnRK2 subclass III was established in Arabidopsis and this mutant lost most of ABA responses such as seed dormancy, germination, post-germination growth, ABA-responsive gene expression and stomata movements (Boudsocq and Lauriere 2005; Fujii and Zhu 2009; Fujita *et al*. 2009; Nakashima *et al.* 2009). Thus SnRK2 subclass III members are considered major positive regulator in ABA signaling.

Ten rice SnRK2 kinases were designated SAPK1 through SAPK10, which stand for osmotic Stress/ABA-activated Protein Kinase (Kobayashi *et al*. 2004, 2005) (**Fig. 5**). SAPK3 is identical with the previously reported REK and SAPK1 and SAPK2 are similar with wheat PKABA1 and Barley HvPKABA1 (Hotta *et al*. 1998; Kobayashi *et al*. 2004). SAPK6 is identical with the OSRK1 induced by dehydration (Chae *et al.* 2007). SAPKs also can be classified into three subgroups. Each subclass has similar activation characteristics with those of Arabidopsis except for Subclass II which is only activated by osmotic stress (Kobayashi *et al*. 2004). Subclass III members including SAPK8, 9 and 10 are activated by ABA and Subclass I were activated by osmotic stress but not by ABA. It was reported that Subclass II members including SAPK1, 2 and 3 were activated by osmotic stress but not by ABA unlike Arabidopsis (Kobayashi *et al*. 2004) (**Fig. 5**). However, it is controversial because SAPK2 was able to activate the OREB1, bzip transcription factor when it is transiently expressed in Arabidopsis protoplast (Kim *et al*. 2012). The amino acid sequences of rice SAPKs and Arabidopsis SnRKs were highly conserved in N-terminal but there are variable regions in C-terminal. The variable region is quite specific to the subclass I, II and III (**Fig. 5**).

Molecular mechanisms of ABA signaling based on the protein structure

The core of the ABA signaling network is consisted of PYL/RCAR, subclass A PP2C and SnRK2. Recently, the structure of each component and their complex were identified by several research groups (Melcher *et al*. 2009; Nishimura *et al*. 2009; Soon *et al*. 2012). Based on the protein structure analysis, gate-latch-lock mechanism is suggested for the PP2C inhibition mechanism by ABA receptors at the molecular level. Two highly conserved loops close by ABA binding pocket plays a role as gate and latch (**Fig. 3**). ABA binding changes the protein structure, which led to the closure of gate on to the latch and make the receptor to interact with the PP2C active site. Thus, this interaction inhibits PP2C activity by blocking the active site from substrate access. Finally, the conserved tryptophan of PP2C active site inserts between the gate and latch to lock the interaction and allow PP2C-PYR complex to be stable. These model solved questions for how ABA receptor PYR/RCAR is able to inhibit the subclass A PP2Cs at the molecular level (Melcher *et al*. 2009).

Recently, the crystal structure of SnRK2.6-HAB1 complex was also determined. Intriguingly, SnRK2.6-HAB1 interface are very similar with PYL-PP2C interface in terms of crystal structure. The activation loop of SnRK2 interacts with an active site of PP2C and the conserved tryptophan of PP2C, which was used to lock PYL-PP2C interaction inserts into the kinase active cleft and completely blocks its activity (Soon *et al*. 2012).

Additionally, acidic motif consisted of about 25 amino acid residues in the C-terminus of SnRK2.6 interact with HAB1. This interaction allows SnRK2.6 to be able to be dislodged from HAB1 by ABA bound PYLs but not to be able to be fully dissociated from HAB1. Thus SnRK2.6 remains to be tethered by its ABA box to the HAB1(Soon *et al*. 2012). These complex protein structures explain how PYL-PP2C-SnRK2 interacts and is activated by ABA at the molecular level.

FUTURE PERSPECTIVES

Climate changes due to increased CO_2 are a challenging issue threatening the survival of human beings on Earth. Many plant biotechnologists think that they can contribute to solve the problem by improving the capacity of crops to survive in adverse environments. ABA signaling represents a good target for improving the abiotic stress tolerance of crops. Alteration of ABA signaling of plants through conventional breeding or transgenic approaches might cause big changes in the responsiveness of crops to abiotic stresses (Cutler *et al*. 2010; Guo *et al*. 2011). PYL/RCAR-mediated ABA signaling seems to be the major pathway for regulation of gene expression and abiotic stress tolerance in the model plant *Arabidopsis thaliana*. In Arabidopsis, constitutive expression of PYL/RCAR led not only to ABA hypersensitive growth responses and drought tolerance (Santiago *et al*. 2009); however, not much has been studied in crop plant and require intensive efforts.

In conclusion, several different ABA receptors are highly conserved in monocot crop plants such as rice when compared with Arabidopsis. Other components of the ABA signaling pathway, such as subclass A PP2C and SnRK2 also seem to be well-conserved in rice (Kim *et al.* 2012). By better understanding the molecular mechanisms of ABA signaling in rice through detail molecular studies would allow us to modulate the responsiveness to abiotic stresses by using traditional breeding or transgenic technologies, which ultimately will help in enhancing the crop productivity and yield.

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