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REVIEW ARTICLE

Mechanism of ABA Signal Transduction: Agricultural Highlights for Improving Drought Tolerance

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Abstract Recent progress has been made in understanding the mechanism of ABA signal transduction based on identification of novel genetic components as well as decoding of signaling networks. This has resulted in a wide range of genetic targets for manipulation of crop genomes to obtain drought-tolerant traits. Early events in ABA signaling involve PYR/PYL/RCAR ABA receptors as well as two phosphatase/kinase enzyme pairs, PP2Cs and SnRK2s, with opposite functions. In the end, a number of transcription factors under the control of SnRK2s activate the ABA-dependent gene expression resulting in drought-resistant responses. Due to the evolutionary conservation of ABA signaling and plant drought stress responses in vascular plants, genes identified as major ABA signaling components in Arabidopsis can be used as targets for genetic manipulation of profitable crop species. Therefore, modulation of genes or application of small compounds that specifically function in ABA signal transduction might offer a unique pathway to addressing the global demand aims for the drought- or water deficiency-resistant crop lines without growth penalty.

Keywords: ABA, Crop, Drought stress, Vegetable, Signal transduction, Small compound

Updates on ABA Signaling Mechanism

The plant stress hormone abscisic acid (ABA) is known to mediate plant responses against diverse environmental stress conditions, including drought, salinity, cold, heat, and ozone stresses. Forward genetic approaches based on the model plant Arabidopsis have revealed how abiotic stresses trigger ABA biosynthesis and ABA delivery to the stressed tissues, followed by initiation of signal transductions to promote immediate cellular responses as well as reprogramming of gene expression patterns for plant survival (Cutler et al. 2010; Kim et al. 2010). As a recent breakthrough, the genes *PYR/PYL/RCARs (PYRABACTIN RESISTANCE/PYR1-LIKE/ REGULATORY COMPONENTS OF ABA RECEPTORS)* were recently shown to code for ABA receptors through chemical genetic and biochemical approaches (Ma et al. 2009; Park et al. 2009). Although *PYR/PYL/RCAR* genes exist as a gene family composed of 14 genes, forward genetic screens to identify mutants resistant to the ABA agonist pyrabactin resulted in identification of *PYR1*, which activates specific subsets of the *PYR/PYL/RCAR* pathways (Park et al. 2009). Additionally, biochemical searches for interactive partners of the major upstream components of the ABA signaling pathway, ABI1 (ABA INSENSITIVE1) and ABI2, led to the identification of RCAR1 and PYR1 (Ma et al. 2009; Nishimura et al. 2010). Major clues regarding the molecular mechanism of ABA perception as well as the regulation between interactive partners such as PP2Cs (Protein phosphatases 2C) and SnRK2s (Sucrose nonfermenting1-related protein kinases 2) have come from structural biology studies (Melcher et al. 2009; Miyazono et al. 2009; Nishimura et al. 2009; Santiago et al. 2009; Yin et al. 2009). Binding of ABA to the pocket of PYR/PYL/RCARs guides the gate motif to the latch domain and this closed lid fits well into the active sites of PP2C enzymes. A PP2C binds to a SnRK2 in the absence of ABA and mediate dephosphorylation of the SnRK2 (Fig. 1). On the other hand, activation of SnRK2s as a critical positive signal for ABA signaling usually begins with autophosphorylation of SnRK2s. Genetic evidence demonstrating that SnRK2.2/2.3/2.6 as major positive regulators of ABA signaling is based on the strong ABA-insensitive phenotypes of the triple mutant *snrk2.2 snrk2.3 snrk2.6* (Fujii and Zhu 2009; Fujita et al. 2009). Furthermore, similar strong ABA-insensitive phenotypes by the sextuple mutant, *pyr1 pyl1 pyl2 pyl4 pyl5 pyl8,* including lack of

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ABA-mediated activation of SnRK2s, indicate that the soluble ABA receptor PYR/PYL/RCAR is a major receptor for the ABA-mediated regulation of SnRK2 kinase activity (Gonzalez-Guzman et al. 2012).

The crystal structure of the SnRK2.6-HAB1 complex demonstrates that the ABA-bound PYL2-HAB1 complex competes with SnRK2.6 for the substrate-binding site of the phosphatase (Ng et al. 2011; Soon et al. 2012). The ABA-induced release of SnRK2.6 from the inhibition of PP2Cs triggers activation of SnRK2.6, which directs the phosphorylation of diverse SnRK2's substrates such as ABF2 (ABA-responsive-element binding factor 2) and ABF3, which are positive regulators of the ABA-induced gene expression (Fujii et al. 2009; Fujita et al. 2009) (Fig. 1).

In addition, ABA-activated SnRK2s regulate guard cell channel activities by phosphorylation of the S-type anion channel, SLAC1 (Geiger et al. 2009; Lee et al. 2009). By activating anion and/or calcium channels, ABA can sequentially induce depolarization of the guard cell membrane, resulting in the outward movement of potassium ions as well as closures of stomatal pores formed by two guard cells (Kim et al. 2010). Besides the phosphorylation by the ABA-activated OST1, SLAC1 is also under the control of calcium-dependent protein kinases, including CPK6, CPK21, and CPK23 (Geiger et al. 2010; Brandt et al. 2012). Transient gene expression studies have shown that whereas CPK23 regulates SLAC1 in a Ca^{2+}-independent manner, CPK21 modulates SLAC1 in a Ca^{2+} concentration-dependent manner. Furthermore, an *ALMT12* mutant was shown to be defective in the ABA-induced stomatal closing as well as produce reduced R-type anion channel currents, suggesting that ALMT12 represents an R-type anion channel, QUAC1 (Meyer et al. 2010). Under drought conditions, ABA activates QUAC1 along with SLAC1 and SLAC1 homologs (SLAHs) for the regulation of stomatal closures and these are controlled by ABI1 and SnRK2.6 (Imes et al. 2013).

ABA-responsive genes are mainly activated by a group of bZIP transcription factors known as ABI5 and ABRE binding factors/ABRE binding proteins (ABF/AREBs), which specifically bind to promoters containing ABA-responsive-elements (ABREs). In addition to SnRK2-mediated phosphorylation of ABF/AREBs, ABRE-dependent gene expression in response to abiotic stress is under the control of other transcription factors, including AP2/ERF, MYB, NAC, NF-Y, and WRKYs. (Antoni et al. 2011; Fujita et al. 2011; Nakashima et al. 2012; Rushton et al. 2012).

Many new approaches have investigated the mechanism of PYR/PYL/RCAR-mediated ABA signal transduction. The small compound, quinabactin/AM1 was recently identified as an effective ABA agonist in vegetative tissues and it activates ABA signal transduction preferentially via the dimeric PYR1 complex (Cao et al. 2013; Okamoto et al. 2013). The proven ability of quinabactin to generate drought-resistant responses in vegetative tissues such as guard cells in soybean and maize as well as in Arabidopsis suggests its possible application in the field for the specific modulation of ABA and drought signaling pathways.

By screening of point mutations that specifically modulate the function of PYL4, recombinant *PYL4(A194T)* was isolated since its overexpression enhanced ABA sensitivities in seed germination, seedling growth, and stomatal regulation (Pizzio et al. 2013). This research demonstrated that isolation of novel alleles or genetic engineering of *PYR/PYL/RCARs* undergoing strong interactions with *PP2Cs* is an effective strategy for improving drought resistance in plants.

Altogether, the detailed characterization of the core components involved in ABA signaling has provided new avenues of genetic manipulation to improve plant drought tolerance.

Agricultural Application of Regulatory Components Involved in ABA Signal Transduction

Agricultural plant production is affected by various kinds of abiotic stress conditions. Especially, due to global climate changes and reduced ground water availability, ensuring sufficient crop production has become an imminent task. Especially, drought stress is one of major abiotic stresses, resulting in up tp 50% of yield reduction. As such, great effort has been undertaken to develop drought-resistant plants *via* breeding or genetic modification to introduce drought tolerant traits into crops (Ashraf 2010; Hadiarto and Tran 2011; Hu and Xiong 2013).

Plants that are exposed to drought stress exhibit growth retardation, perturbation of metabolites including osmotic protective solutes and proteins, reprogramming of gene expression patterns, elevation of reactive oxygen species (ROS) levels, alteration of plant hormone levels, and onset of developmental plasticity compatible to drought conditions (Hu and Xiong 2013). As there is a wide range of physiological resets induced by drought, plant drought resistance is achieved by complex networks of signal transduction via both ABA-dependent and ABA-independent resistant mechanisms. Upon perception of drought stress by plant cells, endogenous ABA levels increase rapidly (Zeevaart 1980) and initiate stress resistance mechanisms. Because ABA-dependent signal transduction is the main pathway involved in drought-tolerant responses, ABA signaling components are regarded as major targets to modify for improving drought tolerant traits. However, there are also ABA-independent pathways supporting drought resistance in plants. ABA-independent pathways involve the transcriptional regulation of genes controlled by promoters containing the

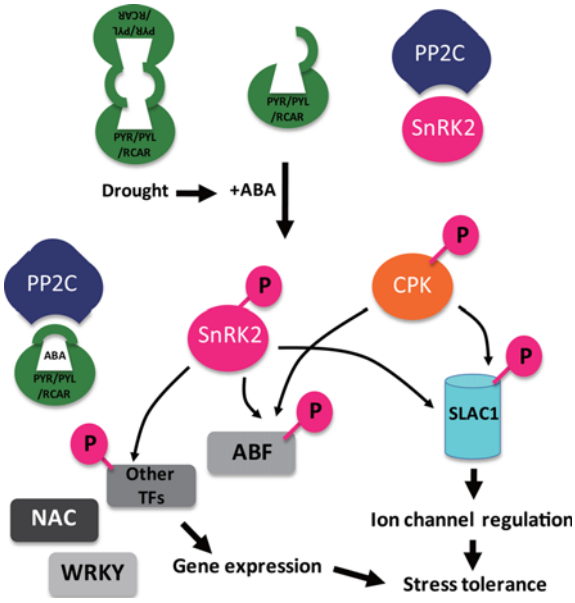

Fig. 1. Summary of ABA signaling pathways that result in drought stress resistant responses. See the text for details.

dehydration-responsive element/C-repeats (DRE/CRTs) and DRE-binding proteins/C-repeat-binding factors (DREBs/CBFs), which bind to DRE/CRTs in response to cold or osmotic stress (Yamaguchi-Shinozaki and Shinozaki 2005; Roychoudhury et al. 2013).

Combined with recent findings on ABA signal transduction in Arabidopsis (Fig. 1), investigation into the genetic regulation of core ABA signal transduction components could promote the introduction of drought tolerance responses into various crop species. Recent reports on the successful application of core ABA signaling components especially from *Oryza sativa*, *Zea mays*, *Triticum aestivum*, *Glycine max*, and *Brassicaceae* along with transgenic characterization of drought stress responses are discussed below (Table 1).

Rice ABA Signal Transduction

As PYR/PYL/RCARs are newly recognized as major ABA perception molecules in Arabidopsis, whether or not PYR/PYL/RCAR orthologs from other plant species function in

Table 1. Recent reports on the transgenic characterization of drought stress responses by modulation of crop ABA signaling components

Species	Gene	Drought tolerance-related plant phenotypes	Reference
Rice (*Oryza sativa*)	*OsPYL11/RCAR5*	Overexpression resulted in enhanced drought responses	Kim et al. (2012)
	OsRK1/SAPK6	Overexpression resulted in reduced ABA responses	Chae et al. (2007)
	OsSLAC1	The *slac1* mutant with increased stomatal conductance	Kusumi et al. (2012)
	OsbZIP16	Overexpression resulted in enhanced drought responses	Chen et al. (2012)
	OsbZIP23	Overexpression resulted in enhanced drought responses	Xiang et al. (2008)
	OsbZIP46CA1	Overexpression resulted in enhanced drought responses	Tang et al. (2012)
	OsbZIP71	Overexpression resulted in enhanced drought responses	Liu et al. (2014)
	OsbZIP72	Overexpression resulted in enhanced drought responses	Lu et al. (2009)
	OsABF1	T-DNA mutants produced drought sensitive responses	Amir Hossain et al. (2010)
	OsNAC5	Overexpression resulted in enhanced drought responses	Takasaki et al. (2010)
	OsNAC6	Overexpression resulted in enhanced drought responses	Takashima et al. (2007)
	OsWRKY45-1	Overexpression produced drought sensitive responses	Tao et al. (2011)
	OsWRKY45-2	Overexpression resulted in enhanced ABA responses	Tao et al. (2011)
Maize (*Zea Mays*)	*ZmCPK11*	ABA-induced ROS production	Ding et al. (2013)
	ZmPP2C (AY621066)	Overexpression produced drought sensitive responses	Liu et al. (2009)
	ZmOST1	Complement the Arabidopsis *ost1-2* stomatal phenotypes	Vilela et al. (2013)
	ZmbZIP72	Overexpression resulted in enhanced drought responses	Ying et al. (2012)
Wheat (*Triticum aestivum*)	*TaSnRK2.4*	Overexpression resulted in enhanced drought responses	Mao et al. (2010)
	TaSnRK2.8	Overexpression resulted in enhanced drought responses	Zhang et al. (2010)
	TaERA1	VIGS of *TaERA1* produced drought tolerant responses	Manmathan et al. (2013)
	TaWRKY2	Overexpression resulted in enhanced drought responses	Niu et al. (2012)
	TaWRKY19	Overexpression resulted in enhanced drought responses	Niu et al. (2012)
	TaNAC2a	Overexpression resulted in enhanced drought responses	Tang et al. (2012)
Soybean (*Glycine max*)	*GmbZIP1*	Overexpression resulted in enhanced drought responses	Gao et al. (2011)
	GmNFYA3	Overexpression resulted in enhanced drought responses	Ni et al. (2013)
	GsWRKY20	Overexpression resulted in enhanced drought responses	Luo et al. (2013)
	GsAPK	Overexpression resulted in reduced ABA responses	Yang et al. (2012)
Cabbage (*Brassica oleracea*)	*BolABI1*	Overexpression resulted in reduced ABA responses	Yuan et al. (2013)
	BolOST1	Complement the Arabidopsis *ost1-2* stomatal phenotypes	Wang et al. (2013)
	BolABI5	Complement the Arabidopsis *abi5-1* phenotypes	Zhou et al. (2013)

the same manner should have been investigated. Overexpression of *OsPYL11/RCAR5* in transgenic rice has been shown to enhance ABA responses in seed germination and seedling growth assays. Further, BiFC and transient gene expression analyses have revealed that OsPYL11/RCAR5 interacts with OsPP2C30 and SAPK2. Although previous studies on rice SnRK2 homologs have shown that only SAPK8, SAPK9, and SAPK10 are activated by ABA (Kobayashi et al. 2004), overexpression of *SAPK2* in protoplasts was shown to induce *OREB1*–dependent gene expression of a luciferase reporter construct containing ABREs in the promoter (Kim et al. 2012). Similarly, overexpression of *OsRK1/SAPK6* in tobacco was shown to promote ABA-insensitive phenotypes during seed germination and root growth (Chae et al. 2007). The reconstitution of rice ABA responses in transient assays with the expression of only core ABA signaling genes has demonstrated that the ABA signaling mechanism in Arabidopsis is also conserved in rice. These results support the strategy to use of ABA signal transduction components in crop genome engineering to achieve drought-tolerant transgenic plants.

The rice ABF/AREB homologs *OsbZIP16* and *OsbZIP23* were shown to be highly induced by drought stress treatment. As a result, *in vivo* functions of *OsbZIP16* and *OsbZIP23* were examined by generation of overexpressing transgenic rice lines and both enhanced drought stress responses in gene expression and seedling growth assays (Xiang et al. 2008; Chen et al. 2012). Consistent with this observation, gene expression of *OsbZIP16* and *OsbZIP23* was induced by exogenous ABA treatment and the overexpressing transgenic rice lines were hypersensitive to ABA treatment. Furthermore, RNA sequencing and microarray analyses of the *OsbZIP16* and *OsbZIP23* transgenic lines found that *OsbZIP16* and *OsbZIP23* regulated drought stress-responsive gene expression.

Similarly, *OsbZIP46* (Tang et al. 2012a), *OsbZIP71* (Liu et al. 2014), and *OsbZIP72* (Lu et al. 2009) were demonstrated to be drought-inducible transcription factors. However, whereas overexpression of *OsbZIP71* and *OsbZIP72* enhanced drought-tolerant and ABA-hypersensitive phenotypes, *OsbZIP46* overexpression in rice produced only ABA-hypersensitive phenotypes without any effect on drought stress responses. As OsbZIP46 contains a transcriptional repressor domain in its central region, a constitutively active form of OsbZIP46, OsbZIP46CA1, was generated by deletion of the repressor domain. Interestingly, overexpression of *OsbZIP46CA1* showed drought-tolerant and ABA-hypersensitive phenotypes. Microarray analysis of the *OsbZIP4CA1* transgenic lines confirmed the induction of stress-responsive genes, which are distinct from those genes induced by *OsbZIP23*. OsbZIP46 were shown to interact with SAPK2, SAPK6, and SAPK9 in yeast two-hybrid and BiFC assays. The specific interactions between the rice ABF and SnRK2 homologs seem to involve phosphorylation events that promote the transcriptional activation of ABA-responsive genes. Consistent with the previous overexpression studies on *bZIP* family genes, T-DNA insertional mutants of the *OsABF1* gene, *osabf1-1* and *abf1-2* showed hypersensitive responses to drought stress and reduced responses in ABA-induction of gene expression in rice (Amir Hossain et al. 2010). Altogether, these data suggest that target gene activation induced by multiple bZIP transcription factors modulates ABA and drought signal transduction in rice. Furthermore, selective and cooperative genetic modification of *bZIP* family genes might help us develop specific drought-tolerant phenotypes in crops.

Overexpression of the drought- and ABA-inducible *OsNAC5* gene in rice has been shown to enhance drought stress responses without any growth defect (Takasaki et al. 2010). Consistently, RNAi-based downregulation of *OsNAC5* has been shown to reduce drought stress resistant responses (Song et al. 2011). However the transgenic rice overexpressing *OsNAC6* were found to display enhanced drought stress tolerance along with growth defect penalties (Nakashima et al. 2007). Therefore, in contrast to OsNAC5, OsNAC6 plays a role in plant growth and development as well as in drought stress signal transduction.

In the case of *OsWRKY45-1* and *OsWRKY45-2* overexpressing transgenic lines, the two similar genes were shown to display opposite phenotypes in response to drought stress (Tao et al. 2011). Specifically, overexpression of *OsWRKY45-1* produced reduced ABA sensitivity and severe drought stress damage, whereas overexpression of *OsWRKY45-2* resulted in enhanced ABA sensitivity with no increase in drought-resistant phenotypes. Considering that *OsWRKY45-1* and *OsWRKY45-2* produced opposite phenotypes to pathogens (Tao et al. 2009), one can conclude that the 10 amino acids difference between these two transcription factors is responsible for the differential regulation of ABA and drought signaling.

Arabidopsis SLAC1 represents a core component of both ABA and CO_2 signaling in guard cells. Previous investigation of rice *SLAC1* homolog gene mutants showed that *SLAC1* from rice functions in the CO_2-mediated regulation of stomatal closures (Kusumi et al. 2012). Although its exact role during drought stress or ABA signaling in rice has not been fully elucidated, *SLAC1* could be a genetic modification target to control drought responses and CO_2 assimilation in the crop model.

Maize ABA Signal Transduction

Functional characterization of the maize *PP2C* gene *ZmPP2C* (AY621066) was performed in Arabidopsis by overexpressing *ZmPP2C* under the control of 35S promoter.

The resulting transgenic Arabidopsis line showed reduction of ABA-induced gene expression as well as ABA-mediated inhibition of seed germination and seedling growth (Liu et al. 2009). Consistently, the *ZmPP2C* overexpression line resulted in hypersensitive responses to drought stress during seed germination and seedling growth. Another critical component of upstream ABA signaling in Arabidopsis, SnRK2.6/OST1 was also examined in maize. Ectopic expression of the maize homolog of *SnRK2.6/OST1*, *ZmOST1,* in Arabidopsis *ost1-2* mutant was able to functionally complement defective mutant guard cell responses to drought stress (Vilela et al. 2013). Yeast two-hybrid screens using ZmOST1 as bait identified ZmSNAC1 as an interaction partner for ZmOST1. In addition, BiFC and in-gel kinase assays demonstrated that osmotic stress-activated ZmOST1 phosphorylated ZmSNAC1. Interestingly, the interaction between ZmOST1 and ZmSNAC1 might induce the ABA-dependent nuclear speckle formation as well as protein degradation of ZmSNAC1. Another possible transcription factor targeted by maize SnRK2s could be ZmbZIP72, as overexpression of *ZmbZIP72* in Arabidopsis has been shown to enhance drought stress-tolerant and ABA-hypersensitive phenotypes (Ying et al. 2012). These data indicate that the maize ABA-PP2C-SnRK2 signaling complex that promotes drought stress resistance might function similarly to the Arabidopsis model.

The maize CDPK ortholog ZmCPK11 was shown to be induced by ABA and H_2O_2 at both the gene expression and kinase activity levels (Ding et al. 2013). Overexpression and RNAi-mediated downregulation of *ZmCPK11* in maize protoplasts showed that ZmCPK11 functions as a positive regulator of ABA-induced ROS production. Moreover, ZmCPK11 was shown to upregulate ZmMPK5, which was isolated as a specific ABA-induced MAP kinase in maize (Ding et al. 2009). The control of ABA signaling by ZmCPK11 illustrates that CPKs and/or MAPKs have critical functions in maize ABA signal transduction similar to their roles in Arabidopsis ABA signaling.

Wheat ABA Signal Transduction

Among the wheat homologs of the major Arabidopsis ABA signaling regulators, *TaSnRK2.4* (Mao et al. 2010), *TaSnRK2.8* (Zhang et al. 2010), *TaERA1* (Manmathan et al. 2013), *TaWRKY2/19* (Niu et al. 2012), and *TaNAC2a* (Tang et al. 2012b) were reported to produce drought-related phenotypes *in planta*. Two wheat *SnRK2* homologs, *TaSnRK2.4* and *TaSnRK2.8,* were functionally tested by overexpression in Arabidopsis, and they showed enhanced-drought tolerant phenotypes (Mao et al. 2010; Zhang et al. 2010). It should be noted that overexpression of *TaSnRK2.4* and *TaSnRK2.8* resulted in increased yields. Moreover, virus-induced gene silencing of the *ERA1* gene in wheat has been shown to produce similar ABA-hypersensitive phenotypes as those observed in Arabidopsis *era1* mutant, including improved water use efficiency and delayed seed germination (Manmathan et al. 2013). Altogether, the general ABA signal transduction mechanism is conserved and is mainly responsible for generation of drought-resistant responses in wheat.

Additionally, two stress-inducible wheat *WRKY* genes, *TaWRKY2* and *TaWRKY19,* were expressed in Arabidopsis and produced drought-resistant phenotypes (Niu et al. 2012). Especially, TaWRKY19 was shown to bind to the promoter of *RD29B*, which is a well-known ABA-inducible gene. This indicates that at least some *WRKY* genes in wheat are directly involved in ABA signal transduction. As overexpression of the *NAC* homolog *TaNAC2a* in tobacco promotes drought resistance and fresh weight increases (Tang et al. 2012b), wheat stress-related transcription factors such as *WRKYs* or *NACs* could be a target for genetic modification of wheat for induction of drought resistance.

Soybean ABA Signal Transduction

The soybean *bZIP* family gene, *GmbZIP1,* which is closely related to *ABF2,* is transcriptionally induced by ABA and drought (Gao et al. 2011). Overexpression of *GmbZIP1* resulted in ABA-hypersensitive and drought-tolerant phenotypes in wheat as well as in Arabidopsis (Gao et al. 2011).

Overexpression of the ABA-inducible *GsWRKY20* gene from wild soybean (*Glycine soja*) in Arabidopsis has been shown to induce tissue-dependent biphasic ABA responses. Specifically, ABA responses were shown to be reduced during seed germination and seedling growth while enhanced in guard cells and vegetative tissues resulting in drought-tolerant phenotypes (Luo et al. 2013). As Nuclear Factor Y (NF-Y) is involved in stress signal transduction, overexpression of the ABA-inducible *GmNFYA3* gene in Arabidopsis exhibited enhanced drought-tolerant and ABA-hypersensitive phenotypes (Ni et al. 2013). Interestingly, mRNA of *GmNFYA3* contains a target site for *miRNA169*. In transient tobacco expression assays, *GmNFYA3* mRNA was shown to be cleaved by *miRNA169*, suggesting that miRNA-mediated regulation of ABA-responsive transcription factors controls ABA and drought stress signal transduction (Sunkar et al. 2007).

The wild soybean homolog of SnRK2, GsAPK was identified as an ABA-activated protein kinase (Yang et al. 2012). Gene expression of *GsAPK* was also induced by ABA and drought stress treatment. Overexpression of *GsAPK* in Arabidopsis has been shown to reduce ABA responses during seed germination and root growth, implying a role in soybean ABA signal transduction.

Cabbage ABA Signal Transduction

Considering the close evolutionary relationship between Arabidopsis and the vegetable crop, cabbage, it is expected to find that major components of ABA and drought signal transduction from Brassica function similarly to those of Arabidopsis.

The OST1 ortholog of *Brassica oleracea* (BolOST1), which is induced by drought or salt stress can specifically interact with both BolABI5 and BolABI1. This implies that the function of BolOST1 might be conserved in cabbage plants, similar to the case of Arabidopsis (Wang et al. 2013). BolABI5 has been shown to complement the ABA-insensitive seed germination phenotypes of Arabidopsis *abi5-1* mutant (Zhou et al. 2013). The negative regulation of ABA signaling by PP2Cs is also conserved in Brassica plants, as overexpression of *BolABI1* has been shown to reduce ABA responses (Yuan et al. 2013).

Conclusion

Since the identification of PYR/PYL/RCARs, a better understanding of signaling networks among the canonical ABA signaling components has shed light on the genetic modification of ABA signal transduction for improving drought tolerance in plants (Fig. 1 and Table 1). Major targets for this purpose include PYR/PYL/RCARs-PP2C-SnRK2s complexes as well as their phosphorylation substrates such as transcription factors or channel proteins.

Granting that interplay between ABRE-mediated and DRE-mediated transcriptional modules is required to fully develop drought-resistant responses, systems biological approaches involving genomics, proteomics, and interactomics are required to properly select targets to initiate, amplify, and maintain the drought stress-tolerant responses without causing any growth defect or diminishing crop production.

Besides the well-established canonical ABA signaling pathways, there have been also reports on the identification of novel genes and pathways involved in ABA signal transduction. Small RNAs such as miRNAs as well as epigenetic control of stress responsive gene promoters (Sunkar et al. 2007; Han and Wagner 2013) could provide new target genes to modulate drought stress responses including stress memory and acclimation to new environments.

There are also concerns over using genetic modification versus relying on classical breeding methods. One of criticism argues that transgenic lines showing drought-tolerant phenotypes should be assessed under field conditions to prove that their drought tolerance established in the laboratory is not merely a delayed stress response mechanism (Lawlor 2013). In addition, public reluctance to accept GM crops on their dining tables could be circumvented by screening small compounds that can target specific components of the ABA signaling pathway in non-transgenic crop plants.

Future research on the application of ABA signal transduction components to genetic modification of crops should focus on field conditions and long-term regulation of drought stress signal transduction.

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