Global Journal of Agricultural Research
Vol.10, No.2, pp.1-12, 2022
Print ISSN: 2053-5805(Print),
Online ISSN: 2053-5813(Online)

Review on Biosynthesis, Signal transduction of ABA and its Function in Physiological and Metabolic Process of Crop plants

Bewket Getachew Bekele
Pawe Agricultural Research Center, Ethiopian Institute of Agricultural Research, Ethiopia

Citation: Bewket Getachew Bekele (2022) Review on Biosynthesis, Signal transduction of ABA and its Function in Physiological and Metabolic Process of Crop plants, *Global Journal of Agricultural Research* , Vol.10, No.2, pp.1-12

ABSTRACT: *Abscisic acid (ABA) is a phytohormone that regulates physiological processes such as seed maturation, seed dormancy, and stress adaptation. These physiological responses are triggered by the fluctuation of endogenous ABA levels in accordance with changing surroundings or developmental stimuli. Endogenous ABA levels are largely controlled by the balance between biosynthesis and catabolism. ABA is produced by cleavage of a 40-carbon carotenoid precursor that is synthesized from isopentenyl diphosphate via the plastid terpenoid pathway. ABA is synthesized in almost all cells that contain plastids and is transported via both the xylem and the phloem. ABA in tissues can be measured by bioassays based on growth, germination, or stomatal closure. Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress.*

KEYWORDS: biosynthesis, signal transduction, ABA, physiological , metabolic process crop plants

INTRODUCTION

Abscisic acid (ABA) is a 15-C weak acid that was first identified in the early 1960s as a growth inhibitor accumulating in abscising cotton fruit ("abscisin II") and leaves of sycamore trees photoperiodically induced to become dormant (reviewed in Cutler *et al*., 2010). ABA has since been shown to regulate many aspects of plant growth and development including embryo maturation, seed dormancy, germination, cell division and elongation, floral induction, and responses to environmental stresses such as drought, salinity, cold, pathogen attack and UV radiation.

Abscisic acid has been found to be a ubiquitous plant hormone in vascular plants. It has been detected in mosses but appears to be absent in several genera of fungi make ABA as a secondary metabolite. Within the plant, ABA has been detected in every major organ or living tissue from the root cap to the apical bud. ABA is synthesized in almost all cells that contain chloroplasts or amyloplasts (Cutler *et al*., 2010).

The plant hormone ABA is involved in several biotic and abiotic responses and is associated with the regulation of complex signal transductions including seed dormancy, growth and development. Several key components compose the ABA signaling pathway and each component plays a role as a positive and negative regulator in each step. Several members of the group A PP2Cs and SnRK2s ABA receptors are known to exist. Thus, many possible interactions can occur and each interaction has the potential to regulate downstream targets (Nakashima K. *et al*., 2009).

Generally, ABA used to manage seed dormancy, germination, flowering, modulation of root architecture, stress responses, stomatal regulation, seedling growth and other physiological and morphological changes of crop plants.

Objective: To review on Biosynthesis, Signal transduction of ABA and its Function in Physiological and Metabolic Process of Crop plants.

LITERATURE REVIEW

Biosynthesis of ABA in Crop Plants

ABA is known to be synthesized via two distinct pathways. One is the direct pathway that occurs in phytopathogenic fungi and the other is the indirect pathway that operates in plants. IPP for the direct pathway is synthesized from the mevalonate (MVA) pathway that exists in prokaryotes and almost all eukaryotes. On the other hand, the indirect pathway uses the methylerythritol phosphate (MEP) pathway as a source of isopentenyl pyrophosphate (IPP). The MEP pathway seems to exist in cyanobacteria and all photosynthetic eukaryotes ancluding crop plants(Schwartz and Zeevaart 2010).

Direct pathway synthesis

In phytopathogenic fungi such as *Botrytis cinerea* and *Cercospora cruenta*, ABA is synthesized from MVA. When radiolabeled MVA or farnesyl diphosphate (FDP) was fed to ABA-producing fungi, the labels were effectively incorporated into ABA. In addition, when [1-13C] glucosewas fed to ABA-producing fungi or plants, the positions of the labeled carbons in ABA were different between plants and fungi, because the labeled carbon was differently incorporated into IPP depending on its source, the MVA or MEP pathway (Akira *et al*., 2014). These results strongly indicate that fungal ABA is synthesized from IPP produced in the MVA pathway. Since all intermediates between FDP and ABA are sesquiterpenes, the ABA biosynthetic pathway in fungi has been referred to as the direct pathway (Nambara and Marion-Poll 2005). The direct pathway involves several modifications of FDP to generate ABA Isomers of ionylideneethanol and/or ionylideneacetic acid have been identified from several fungi and are supposed to be the endogenous precursors of ABA in fungi (Akira *et al*., 2014).

Indirect pathway synthesis

ABA was discovered in the 1960s and subsequently xanthoxin (Xan) was isolated as a plant growth inhibitor like ABA. Xan had structural similarity to part of an epoxycarotenoid. A conversion experiment from Xan to ABA using cell-free extracts from various plant species indicated that 2-*cis*, 4-*trans*-Xan was the possible precursor of ABA that originates from 9-*cis*-epoxycarotenoids (Akira *et al*., 2014).

The *Arabidopsis chloroplasts altered 1* (*cla1*) mutant has a defect in the synthesis of 1-deoxy-d-xylulose-5-phosphate in the MEP pathway and presents carotenoid deficiency resulting in decreased levels of ABA (Estevez et al. 2001). These facts support that C15 ABA is synthesized via C40 epoxycarotenoids that are composed of IPP from the MEP pathway in plants. Therefore, this ABA biosynthetic pathway is referred to as the indirect pathway since C40 epoxycarotenoids are the intermediates of ABA in contrast to the direct pathway in fungi. The indirect pathway has been revealed by biochemical and molecular genetic studies of ABA-deficient mutants. Mutants impaired in carotenoid biosynthesis or molybdenum cofactor (MoCo) synthesis present ABA deficiency as part of a pleiotropic phenotype. The epoxidation steps of zeaxanthin are set as the starting point of the indirect pathway.

Zeaxanthin epoxidase (ZEP) converts zeaxanthin into violaxanthin via antheraxanthin by a two-step epoxidation. Zeaxanthin can be also produced from violaxanthin by violaxanthin de-epoxidase (VDE). This cyclic reaction is called the xanthophylls cycle and is involved in nonphotochemical quenching for photoprotection (Li et al. 2009).

The early steps of ABA biosynthesis take place in plastids and begin with the MEP pathway. An Arabidopsis albino mutant, *chloroplasts altered-1* (*cla1:* AT4G15560), is defective in 1-deoxy-D-xylulose5-phosphate synthase (DXS), the first enzyme of the MEP pathway. The next major phase of ABA biosynthesis is production of carotenoids. Sequential condensation reactions catalyzed by geranyl geranyl diphosphate synthase (GGPPS: AT4G36810) add one isoprene unit at a time to successively generate C10, C15 and C20 molecules (geranyl diphosphate, farnesyl diphosphate, and geranyl geranyl diphosphate (GGPP), respectively). Subsequent head to head condensation of two GGPPs by phytoene synthase (AT5G17230) produces the C40 skeleton that will become phytoene, the first committed carotenoid. Phytoene is subjected to four consecutive desaturation (dehydrogenation) reactions that lead to the formation of lycopene. These reactions are catalyzed by two homologous enzymes: phytoene desaturase (AT4G14210) and -carotene desaturase (AT3G04870) (reviewed in Concepcion, 2012).

The final plastid-localized steps in ABA synthesis are conversion to another C40 compound, trans-neoxanthin, isomerization of either (trans)-violaxanthin and trans-neoxanthin to their 9-cisisomers, and cleavage by 9-*cis*-epoxycarotenoid dioxygenase (NCED) to release the 15C compound xanthoxin, also known as xanthoxal. Neoxanthin synthesis was recently found to

depend on the product of the *ABA4* locus (AT1G67080), a highly conserved unique plastid membrane-localized protein (North et al., 2007).

Signal Transduction of ABA in Crop Plants

Abscisic acid is a key endogenous messenger in plants, and it has a crucial role in various plant stresses. Therefore, understanding the signaling mechanism of ABA is critical for improving crop performance under stress environments, seed dormancy and germination modulation of root architecture, stomatal regulation , seedling growth and others. There are three core components of ABA signaling; pyrabactin resistance (PYR)/pyrabactin resistance-like (PYL)/regulatory component of ABA receptors (RCAR), protein phosphatase 2C (PP2C: acts as negative regulators) and (Sucrose non-fermenting) SNF1- related protein kinase 2 (SnRK2: acts as positive regulators). In the presence of ABA, PYR/PYL/RCAR-PP2C complex formation leads to inhibition of PP2C activity, which allows the activation of SnRK2. Activated SnRK2 then phosphorylates downstream substrate proteins such as transcription factors, and thus facilitating transcription of ABA-responsive genes (Nishimura et al., 2010).The structural and molecular studies conducted by many scientists have convincingly shown that PYR/PYL/RCAR receptors play a central role in ABA perception (Melcher et al., 2010).

PP2C: The negative regulators

Reversible protein phosphorylation catalyzed by protein kinases and phosphatases play a significant role in cellular signal transduction, which helps in the transmission of signals from external to the internal environment of the cell. There are 112 phosphatases encoded in the *Arabidopsis* genome, among them 76 genes encode for PP2Cs (Schweighofer et al., 2004). At least 6 of the nine members of the group A PP2Cs have been shown to involved in ABA signaling, among them, ABI1, ABI2, and HAB1 are the well characterized. The members of the group A have defined roles in different tissues with tissue-specific expression patterns (Ng *et al*., 2014). The PP2C group A genes first characterized are *ABI1* and *ABI2*, which was isolated from a genetic screen in *Arabidopsis*. Both *abi1* and *abi2* mutants show insensitivity in various tissues, developmental stages, suggesting that they act as a negative regulator of ABA signaling.

Both of these mutants display reduced seedling growth, seed dormancy, drought tolerance and stomatal regulations (Yoshida T. et al., 2006). Few years later, HAB1 (homolog to ABI1) and HAB2 were isolated based on their sequence similarity to ABI1 (Saez et al., 2004). Similarly, other members of A group PP2Cs such as AHG1 and AHG3/AtPP2CA were identified in genetic screens of *Arabidopsis* and yeast complementation test. It has been shown that all of the loss of-function mutants of group A PP2Cs exhibit significant ABA hypersensitivity, which establishes them as the major negative regulators of ABA signaling (Antoni et al., 2012).

ABI1, ABI2, and HAB1 showed a dominant role in ABA signaling pathways in both seeds and vegetative tissues; the evidence is supported by gene expression data and genetic analysis.

Recessive *hab1-1* mutants showed enhanced ABA-responsive gene expression, increased ABA-mediated stomatal closure and ABA-hypersensitivity in seed germination. These results indicate that HAB1 negatively regulates ABA signaling (Hirayama and Shinozaki, 2007). These results are consistent with the fact that PP2Cs function as negative regulators of ABA signaling (Mehrotra et al., 2014).

Recently, Singh et al. (2015) reported a group A PP2C from rice gene (*OsPP108*), whose expression is highly inducible under salt and drought stresses. The over expression of *OsPP108* confers ABA insensitivity and tolerance to stresses like high salt and mannitol.

SnRK2 protein kinases: The positive regulators

The identification of PP2Cs showed that reversible protein phosphorylation process is primary in ABA signaling pathway. Protein kinases are known to be involved in ABA signaling pathway. The sucrose non-fermenting 1 (SNF1)-related protein kinase2 (SnRK2) family of protein kinases are plant specific serine/threonine kinases participating in plant response and has a central role in cellular responses to drought and dehydration (Saruhashi et al., 2015). The first protein kinase having ABA-stimulated catalytic activity was identified as an AAPK in *Vicia faba*. AAPK is a positive regulator of ABA-induced stomatal closure. Subsequently, the AAPK ortholog in *Arabidopsis*, OST1 (Open Stomata 1)/SnRK2.6, was shown to function in ABA-mediated stomatal regulation (Yoshida et al., 2002). Similar to AAPK, ABA-activated protein kinases have been identified in *Arabidopsis* (Boudsocq et al., 2004) and rice (Kobayashi et al., 2004); they belong to the SnRK2 family. These ABA-activated protein kinases mediate ABA signaling by phosphorylating their substrate proteins such as transcription factors, ion channels, and metabolic enzymes (Fujita et al., 2013).

Among them, SnRK2.2, SnRK3.3, and SnRK2.6 (Subgroup-III) are known as primary regulators of ABA since they exhibit the strongest activation of ABA. The first member of the *Arabidopsis* SnRK2 family is OST1/SnRK2.6 which identified by forward genetic approach and act as a regulator of ABA signaling. The mutant *snrk2.6* (*ost1*) cannot exhibit ABA-induced stomatal closure, indicating that it positively regulates the stomatal response to ABA. The *SnRK2.6* gene mainly expressed in guard cells, and the vascular system whereas seed germination and post-germination growth were not affected in *snrk2.6* loss-of-function mutant, which indicated that the SnRK2.6 protein kinase may not function in seed germination (Yoshida et al., 2002).

Moreover, SnRK2.2 and SnRK2.3 function redundantly in ABAinduced inhibition of seed germination and post-germination growth. According to Fujii et al. (2007), *snrk2.2* and *2.3* single mutants show weak, while *snrk2.2* and *snrk2.3* double mutant exhibits high ABA-insensitive phenotypes regarding seed germination and seedling growth. The triple mutation (*snrk2.2/2.3/2.6*), nearly block all the main ABA responses.

Quantitative analysis confirmed that ABA treatment increases the phosphorylation of the site *in vivo* and phosphorylation of the site is also increased by osmotic stress (Vlad et al., 2010). SnRK2 activation loop phosphorylation may also implicate unidentified upstream kinases in plants. ABA- or stress-activated SnRK2 family members have been found in *Vicia faba*, soybean, *Arabidopsis*, tobacco, rice, *Chlamydomonas reinhardtii*, maize, sorghum, and wheat (Mao et al., 2010). Understanding how SnRK2 kinases exert their effects in ABA signaling network requires knowledge of SnRK2 targets.

Function of ABA in Physiological and Metabolic Process of Crop Plants.
ABA is generally used to regulate Seed Dormancy and Germination, Stress responses, Stomatal regulation, Seedling growth, pathogen resistance, flowering and others.

Seed dormancy and germination
Dormancy maintenance is correlated with *de novo* synthesis of ABA during imbibition, primarily in the endosperm (Lee *et al*., 2010), whereas release from dormancy largely reflects increased ABA catabolism in both embryos and endosperm by CYP707A2 (Okamoto et al., 2006) and increased GA synthesis dependent on increased expression of GA3ox1 (AT1G15550) and GA3ox2 (AT1G80340) and decreased expression of the GA inactivating enzyme GA2ox (reviewed in Seo *et al*., 2009).

Evidence of cross-talk between auxin and ABA effects on germination is provided by the observations that exogenous auxin enhances the inhibitory effects of ABA, and that the auxin response factor ARF10 (AT2G28350) promotes ABA sensitivity (Liu *et al*., 2007). ARF10 is subject to post-transcriptional control by miRNA160, and this down regulation is critical to permit germination. Cytokinin promotes germination, and this effect is antagonized by ABA inhibition of cytokinin biosynthesis by reducing expression of the main isopentenyl transferase genes expressed in seeds (Wang *et al*., 2011).

Strigolactones have recently been found to alleviate thermoinhibition of germination by reducing the ABA:GA ratio, in part by reducing expression of ABA biosynthetic enzymes such as NCED9 (Toh *et al*., 2012).

Stress responses
A critical function of ABA during vegetative growth is to mediate response to environmental stresses such as drought, salinity, and cold (reviewed in Huang *et al*., 2012). These stresses are similar in that they all impose cellular osmotic and oxidative stress, but they differ in other effects and consequently the appropriate responses are not identical. ABA is also implicated in response to flooding induced hypoxic stress, which leads to reduced ABA levels in submerged tissues and increased ABA in shoots of flooded plants (Hsu *et al*., 2011). This might explain the earlier observation that exogenous ABA promoted tolerance of hypoxic stress in roots, but

not shoots, in that shoots may have already responded by making sufficient ABA to be beneficial.

At the whole plant level, slightly elevated ABA levels (characteristic of mild water stress conditions) promote root growth but inhibit shoot growth, leading to an increased root:shoot ratio. These roots respond to moisture gradients by positive "hydrotropism" (reviewed in Moriwaki et al., 2012).

Genetic studies have shown that drought rhizogenesis is regulated by ABA, auxin and some gibberellins, and disrupted in the ABA-insensitive mutant *abi1-1*, but not in *abi2-1* or *abi3* mutants. The flow of water across cell membranes to regulate growth and transpiration is largely controlled by aquaporins present in the tonoplast and plasma membranes. Aquaporin content and activity are regulated by many factors, including effects on transcription, protein stability, subcellular trafficking, and gating by changes in phosphorylation, pH or Ca2+ (reviewed in Maurel et al., 2008). Arabidopsis encodes 13 plasma membrane localized isoforms (PIPs) and 10 tonoplast localized aquaporins (TIPs), which display differential organ/tissue/cell-specificities, and different responses to stress or ABA treatment (Alexandersson et al., 2005).

Stomatal regulation

In another important response to drought stress, ABA regulates the transpiration rate via effects on stomatal aperture both by promoting closure and inhibiting opening (reviewed in Joshi-Saha *et al*., 2011). Although both effects result in closed stomata, they are not simple reversals of the same process in that they involve different ion channels regulated by different signaling mechanisms. In addition to this local control of guard cell physiology, recent studies suggest that ABA affects stomatal conductance by reducing hydraulic conductance of leaf vascular tissues, possibly by decreasing bundle sheath aquaporin expression or activity (Pantin *et al*., 2013). Surprisingly, many mutants that have defects in guard cell response to ABA are still responsive to hydraulic conductance and humidity effects on stomatal conductance (Pantin et al., 2013). However, extremely high ABA concentrations (400 μM) were used to provoke stomatal closure in the ABA-insensitive mutants (Pantin *et al*., 2013), which might account for the discrepancy with the initial observation that 10 μM exogenous ABA intensified withering of stems and siliques of the *abi1-1* and *abi2-1* mutants.

ABA perception in guard cells is mediated by multiple receptors. Several members of the PYR/PYL/RCAR receptor family mediate intracellular perception (Gonzalez-Guzman *et al*., 2012), whereas the GTG receptors have been implicated in perception at the plasma membrane (Pandey et al., 2009), although their function and localization is controversial. The plastid localized CHLH protein also appears to regulate stomatal response to ABA, but its identity as a receptor is also controversial (Tsuzuki *et al*., 2011).

Seedling growth

The relative importance of the various family members of the core ABA signaling pathway changes after germination, in part reflecting differences in expression. The receptors that are most highly expressed in most developmental stages are PYR1, PYL1 (AT5G46790), PYL4 (AT2G38310), PYL8 (AT5G53160), PYL5 (AT2G40330), and PYL2 (AT2G26040) (Gonzalez-Guzman *et al*., 2012).

Among these, PYR1 is consistently highly expressed, but the others vary among stages and organs. At the tissue level, further variation is seen. Sextuple mutants knocking out all of these genes are highly resistant to ABA effects on many aspects of growth, but lower order mutants show different degrees of resistance depending on which and how many genes are mutant.

Downstream of the SnRK2s, the bZIP transcription factors also change in relative abundance such that seedling growth is controlled more by the ABFs/AREBs than by ABI5 (Finkelstein *et al*., 2005). ABA plays another role in nutrient signaling in seedlings by mediating the regulatory effects of nitrate on root branching (Signora *et al*., 2001). More recently it has become clear that there is substantial cross-talk between nutrient-based and hormonal signaling (Krouk *et al*., 2011). Reciprocally, auxin, cytokinins, ethylene and ABA regulate nitrogen uptake and assimilation, creating a cycle in which nutrients control hormone levels and consequently growth andnutrient use. Root branching, which directly affects access to soil nutrients, is regulated by soil nitrogen and phosphate levels, as well as by interactions between auxin, cytokinin and ABA signaling (ShkolnikInbar and Bar-Zvi, 2010).

ABA also induces distinct concentration-dependent effects on primary root growth: growth is promoted at nanomolar ABA levels, but inhibited at micromolar levels. Mechanistically, the promotion has been ascribed to decreased cell division in the quiescent center (QC) and suppressed differentiation of stem cells, collectively resulting in meristem maintenance and continued growth (Zhang *et al*., 2010).

REFERENCE

Akira Endo, Masanori Okamoto and Tomokazu Koshiba, 2014. ABA Biosynthetic and Catabolic Pathways. Crop Breeding Research Division,NARO Hokkaido Agricultural Research Center, Sapporo, Japan.

Alexandersson, E., Fraysse, L., Sjovall-Larsen, S., Gustavsson, S., Fellert, M., Karlsson, M., Johanson, U., Kjellbom, P., 2005. Whole gene family expression and drought stress regulation of aquaporins. Plant Mol. Biol. 59: 469-484.

Antoni, R., Gonzalez-Guzman, M., Rodriguez, L., Rodrigues, A., Pizzio, G. A., and Rodriguez, P. L.,2012. selective inhibition of clade A phosphatases type 2C by PYR/PYL/RCAR abscisic acid receptors. *Plant Physiol.* 158, 970–980. doi: 10.1104/pp.111.188623

Concepcion M., 2012. Carotenoid biosynthesis in Arabidopsis: A colorful pathway. Vol. 10. The Arabidopsis Book; p. e0158. doi:10.1199/tab.0158.

Cutler S.R., Rodriguez P.L., Finkelstein R.R., Abrams S.R., 2010. Abscisic acid: Emergence of a core signaling network. Ann. Rev. Plant Biol. 61:651–679.

Cutler S.R., Rodriguez P.L., Finkelstein R.R., Abrams S.R., 2010. Abscisic acid: Emergence of a core signaling network. Ann. Rev. Plant Biol. 61:651–679.

Estevez JM, Cantero A, Reindl A, Reichler S, Leon P., 2001. 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J Biol Chem. 276:22901–9.

Finkelstein, R., Gampala, S.S.L., Lynch, T.J., Thomas, T.L., Rock, C.D., 2005. Redundant and distinct functions of the ABA response loci *ABAINSENSITIVE(ABI)5* and *ABRE BINDING FACTOR (ABF)3*. Plant Mol. Biol. 59: 253-267.

Fujii, H., Verslues, P. E., and Zhu, J. K., 2007. Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. *Plant Cell* 19, 485–494. doi: 10.1105/tpc.106.048538

Fujita, Y., Yoshida, T., and Yamaguchi-Shinozaki, K., 2013. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiol. Plant* 147, 15–27. doi: 10.1111/j.1399-3054.2012.01635.x

Gonzalez-Guzman, M., Pizzio, G.A., Antoni, R., Vera-Sirera, F., Merilo, E., Bassel, G.W., Fernández, M.A., Holdsworth, M.J., Perez-Amador, M.A., Kollist, H., Rodriguez, P.L., 2012. Arabidopsis PYR/PYL/RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. Plant Cell 24: 2483–2496.

Hirayama, T., and Shinozaki, K., 2007. Perception and transduction of Abscisic acid signals: keys to the function of the versatile plant hormone ABA. *Trends Plant Sci.* 12, 343–351. doi: 10.1016/j.tplants.2007.06.013

Hsu, F.-C., Chou, M.-Y., Peng, H.-P., Chou, S.-J., Shih, M.-C., 2011. Insights into hypoxic systemic responses based on analyses of transcriptional regulation in Arabidopsis. PLoS ONE 6: e28888.

Huang, G.-T., Ma, S.-L., Bai, L.-P., Zhang, L., Ma, H., Jia, P., Liu, J., Zhong, M., Guo, Z.-F., 2012. Signal transduction during cold, salt, and drought stresses in plants. Mol. Biol. Rep. 39: 969-987.

Joshi-Saha, A., Valon, C., Leung, J., 2011. Molecular mechanisms of abscisic acid action in plants and its potential applications to human health. *In* I Turkan, ed, Plant Responses to Drought and Salinity Stress: Developments in a Post-Genomic Era, Vol 57. Academic Press LtdElsevier Science Ltd, London, pp 249-292.

Kobayashi, Y., Yamamoto, S., Minami, H., Kagaya, Y., and Hattori, T., 2004. Differential activation of the rice sucrose nonfermenting1-related protein kinase2 family by hyperosmotic stress and abscisic acid. *Plant Cell* 16, 1163– 1177. doi: 10.1105/tpc.019943

Krouk, G., Ruffel, S., Gutierrez, R.A., Gojon, A., Crawford, N.M., Coruzzi, G.M., Lacombe, B., 2011. A framework integrating plant growth with hormones and nutrients. Trends Plant Sci. 16: 178-182

Lee, S.-J., Park, J., Lee, M., Yu, J.-H., Kim, S., 2010. Isolation and functional characterization of CE1 binding proteins. BMC Plant Biol. 10: 277.

Li Z, Wakao S, Fischer BB, Niyogi KK., 2009. Sensing and responding to excess light. Annu Rev Plant Biol. 60:239–60.

Liu, P.-P., Montgomery, T.A., Fahlgren, N., Kasschau, K.D., Nonogaki, H., Carrington, J.C., 2007. Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. Plant J. 52: 133-146.

Mao, X., Zhang, H., Tian, S., Chang, X., and Jing, R., 2010. TaSnRK2.4, *an SNF*1- type serine/threonine protein kinase of wheat (*Triticum aestivum* L.), confers enhanced multi stress tolerance in *Arabidopsis*. *J. Exp. Bot.* 61, 683–696. doi:10.1093/jxb/erp331

Maurel, C., Verdoucq, L., Luu, D.-T., Santoni, V., 2008. Plant aquaporins: Membrane channels with multiple integrated functions. Ann. Rev. Plant Biology 59: 595-624.

Mehrotra, R., Bhalothia, P., Bansal, P., Basantani, M. K., Bharti, V., and Mehrotra, S., 2014. Abscisic acid and abiotic stress tolerance - different tiers of regulation. *J. Plant Physiol.* 171, 486–496. doi: 10.1016/j.jplph.2013.12.007

Melcher, K., Zhou, X. E., and Xu, H. E., 2010. Thirsty plants and beyond: structural mechanisms of abscisic acid perception and signaling. *Curr. Opin. Struct. Biol.* 20, 722–729. doi: 10.1016/j.sbi.2010.09.007

Moriwaki, T., Miyazawa, Y., Kobayashi, A., Takahashi, H., 2012. Molecular mechanisms of hydrotropism in seedling roots of *Arabidopsis thaliana* (Brassicaceae). Am. J. Bot. 100: 25-34.

Nakashima K., Fujita Y., Kanamori N., Katagiri T., Umezawa T., Kidokoro S., Maruyama K., Yoshida T., Ishiyama K., Kobayashi M., et al., 2009. Three *Arabidopsis* SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. Plant Cell Physiol. 50:1345–1363. doi: 10.1093/pcp/pcp083.

Ng, L. M., Melcher, K., Teh, B. T., and Xu, H. E., 2014. Abscisic acid perception and signaling: structural mechanisms and applications. *Acta Pharmacol. Sin.* 35, 567–584. doi: 10.1038/aps.2014.5

Nishimura, N., Sarkeshik, A., Nito, K., Park, S.Y., Wang, A., Carvalho, P.C., Lee, S., Caddell, D.F., Cutler, S.R., Chory, J. and Yates, J.R., 2010. PYR/PYL/RCAR family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. *The Plant Journal*, *61*(2), pp.290-299.

North H.M., Almeida A.D., Boutin J-P., Frey A., To A., Botran L., Sotta B., Marion-Poll A., 2007. The Arabidopsis ABA-deficient mutant *aba4* demonstrates that the major route for stress-induced ABA accumulation is via neoxanthin isomers. Plant J. 50:810–824.

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T., Nambara, E.,2006. *CYP707A1* and *CYP707A2*, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol. 141: 97-107.

Pandey, S., Nelson, D.C., Assmann, S.M., 2009. Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell 136: 136- 148.

Pantin, F., Monnet, F., Jannaud, D., Costa, J.M., Renaud, J., Muller, B., Simonneau, T., Genty, B., 2013. The dual effect of abscisic acid on stomata. New Phytol. 197: 65-72,

Saez, A., Apostolova, N., Gonzalez-Guzman, M., Gonzalez-Garcia, M. P., Nicolas, C., Lorenzo, O., et al., 2004. Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. *Plant J.* 37, 354–369. doi: 10.1046/j.1365-313X.2003.01966.x

Saruhashi, M., Ghosh, T.K., Arai, K., Ishizaki, Y., Hagiwara, K., Komatsu, K., Shiwa, Y., Izumikawa, K., Yoshikawa, H., Umezawa, T. and Sakata, Y., 2015. Plant Raf-like kinase integrates abscisic acid and hyperosmotic stress signaling upstream of SNF1-related protein kinase2. *Proceedings of the National Academy of Sciences*, *112*(46), pp.E6388-E6396.

Schwartz SH, Zeevaart JAD., 2010. Abscisic acid biosynthesis and metabolism. In: Davies PJ, editor.Plant hormones. 3rd ed. New York: Springer; p. 137–55.

Schweighofer, A., Hirt, H., and Meskiene, I., 2004. Plant PP2C phosphatases: emerging functions in stress signaling. *Trends Plant Sci.* 9, 236–243. doi: 10.1016/j.tplants.2004.03.007

Seo, M., Nambara, E., Choi, G., Yamaguchi, S., 2009. Interaction of light and hormone signals in germinating seeds. Plant Mol. Biol. 69: 463-472.

Shkolnik-Inbar, D., Bar-Zvi, D., 2010. ABI4 mediates abscisic acid and cytokinin inhibition of lateral root formation by reducing polar auxin transport in Arabidopsis. Plant Cell 22: 3560-3573.

Signora, L., Smet, I., Foyer, C., Zhang, H., 2001. ABA plays a central role in mediating the regulatory effects of nitrate on root branching in Arabidopsis. Plant J. 28: 655-662.

Singh, A., Jha, S. K., Bagri, J., and Pandey, G. K., 2015. ABA inducible rice protein phosphatase 2C confers ABA insensitivity and abiotic stress tolerance in *Arabidopsis*. *PLoS ONE* 10:e0125168. doi: 10.1371/journal.pone.0125168

Toh, S., Kamiya, Y., Kawakami, N., Nambara, E., McCourt, P., Tsuchiya, Y., 2012. Thermoinhibition uncovers a role for strigolactones in Arabidopsis seed germination. Plant Cell Physiol. 53: 107-117.

Tsuzuki, T., Takahashi, K., Inoue, S.-I., Okigaki, Y., Tomiyama, M., Hossain, M., Shimazaki, K,-I., Murata, Y., Kinoshita, T., 2011. Mg-chelatase H subunit affects ABA signaling in stomatal guard cells, but is not an ABA receptor in *Arabidopsis thaliana*. J. Plant Res. 124: 527-538.

Vlad, F., Droillard, M. J., Valot, B., Khafif, M., Rodrigues, A., Brault, M., et al., 2010. Phospho-site mapping, genetic and in planta activation studies reveal key aspects of the different

phosphorylation mechanisms involved in activation of SnRK2s. *Plant J.* 63, 778–790. doi: 10.1111/j.1365-313X.2010.04281.x

Wang, Y., Li, L., Ye, T., Zhao, S., Liu, Z., Feng, Y.-Q., Wu, Y., 2011. Cytokinin antagonizes ABA suppression to seed germination of Arabidopsis by downregulating ABI5 expression. Plant J. 68: 249-261.

Yoshida, R., Hobo, T., Ichimura, K., Mizoguchi, T., Takahashi, F., Aronso, J., et al., 2002. ABA activated SnRK2 protein kinase is required for dehydration stress signaling in *Arabidopsis*. *Plant Cell Physiol.* 43, 1473–1483. doi: 10.1093/pcp/pcf188

Yoshida, R., Umezawa, T., Mizoguchi, T., Takahashi, S., Takahashi, F., and Shinozaki, K.,2006. The regulatory domain of SRK2E/OST1/SnRK2.6 Interacts with ABI1 and integrates abscisic acid (ABA) and osmotic stress signals controlling stomatal closure in *Arabidopsis*. *J. Biol. Chem.* 281, 5310–5318. doi: 10.1074/jbc.M509820200