ABSTRACT

The plant hormone abscisic acid (ABA) plays a crucial role in the control of the stress response and the regulation of plant growth and development. ABA binding to PYR/PYL/RCAR intracellular receptors leads to inhibition of clade A PP2Cs such as ABI1 or HAB1, causing the activation of the ABA signaling pathway. To obtain further insights into ABA signaling we have focused on the characterization of members of these two families of proteins. We have generated a mutated version of *HAB1* carrying a mutation in the tryptophan-385, which is a key residue for the interaction with the receptor's gating loops and ABA molecule. As a result, hab1^{W385A} was found to be refractory to inhibition by the PYR/PYL/RCAR proteins. Thus, using *in vitro* kinase assays we found that hab1^{W385A} was able to dephosphorylate OST1 even in the presence of ABA and the receptors. *hab1^{W385A}* and *hab1^{G246D}* can be classified as hypermorphic dominant mutations. hab1^{G246D} shows impaired phosphatase activity, whereas the new dominant allele shows wild type activity. Transgenic Arabidopsis lines overexpressing *hab1^{W385A}* showed strong dominant ABA insensitivity. We also analyzed the role of the clade A PP2Cs belonging to the PP2CA branch in the ABA signaling pathway. The generation of the double mutant *pp2ca-1hai1-1,* which shows enhanced ABA sensitivity compared to wild type and the single mutants, revealed that HAI1 is a negative regulator of ABA signaling pathway. Subcellular localization experiments showed that both HAI1 and PP2CA were localized to the nucleus, but also in cytosol and microsomes. Three members of the PP2CA branch, *i.e*.: PP2CA, AHG1 and HAI1, showed selective inhibition by the different PYR/PYL/RCARs. These results suggest that the PYR/PYL/RCAR receptors can discriminate between members of clade A phosphatases. *pyl8* is the only single mutant that shows reduced sensitivity to ABA in root growth assays. GUS reporter analyses showed that PYL8 was present in stele cells, epidermis, columella and lateral root cap and quantification of GUS activity in root showed that *PYL8* is one of the receptors with higher expression levels in this organ. The root tip plays a crucial role for hydrotropism and ABA is a phytohormone involved in this response. The study of the hydrotropic response of combined mutants of both PP2Cs and PYR/PYL/RCARs revealed that the ABA core pathway regulates root hydrotropism. Thus, while the sextuple mutant *pyr/pyl112458* showed reduced root curvature under a moisture gradient, the quadruple mutant of the PP2Cs (*Qabi2-2*) showed a stronger curvature under these conditions, getting away from areas with low water potential better than the wild type. Finally, the last section of this work was focused on exploring new chemical tools to increase drought resistance. We have performed a chemical genetic approach directed to isolate new ABA agonists. Based on structural data of ABA receptors, 500 compounds were selected and assayed in *Arabidopsis*. From these, the compound called 2C06 inhibited root growth in wild type more than in *pyr/pyl/rcar* ABA insensitive mutants and it showed promising *in vitro* results to inhibit PP2Cs and interact with them in Y2H assays.