Unnatural agrochemical ligands for engineered abscisic acid receptors

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Abstract

Existing agrochemicals can be endowed with new applications through protein engineering of plant receptors. A recent study shows an engineered PYR1 ABA receptor can be activated by mandipropamid. Plants engineered with such PYR1 variant are responsive to this agrochemical, which confers protection against drought through activation of ABA signaling.

Keywords: agrochemical, engineered ABA receptor, unnatural ligand, orthogonal ligand-receptor.

Text

Plant growth is severely impaired by adverse environmental conditions such as drought, salinity, cold or high temperature, which can reduce average productivity of crops by 50-80%. In particular, the limited water availability is the single most important factor that reduces crop yield and 70% of fresh water is currently used by agriculture globally [1]. Therefore, engineering plants with enhanced drought tolerance is expected to have a major impact leading to more sustainable cropping systems worldwide. Scientists sometimes claim that drought stress is as complicated to plant biology as cancer is to mammalian biology. However, decades of research focused to elucidate the molecular mechanisms that govern plant adaptive responses to drought have been fruitful in providing molecular insights into the problem. Moreover, it has been demonstrated that plants are amenable to improved stress tolerance through several mechanisms of action. For instance, it is widely recognized that the phytohormone abscisic acid (ABA) represents a crucial signal for plant response to drought. ABA plays a pivotal role to mount and coordinate the drought adaptive response since ABA signaling leads to both regulation of plant water use and adaptive transcriptional responses to avoid or resist drought conditions.

A major breakthrough in ABA perception and signaling occurred in 2009 with the discovery of the PYR/PYL/RCAR family of ABA receptors and the in vitro reconstitution of the pathway. ABA elicits plant responses through binding to soluble PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) receptors, which constitute a multigene family. PYR/PYL/RCAR receptors perceive ABA intracellularly and as a result, form ternary complexes with clade A protein phosphatases type 2C (PP2Cs), thereby inactivating them [2,3]. This prevents the PP2C-mediated dephosphorylation of ABA-activated sucrose non-fermenting 1-related protein kinases (SnRKs) subfamily 2 (SnRK2s), which results in the activation of a SnRK2-dependent phosphorylation cascade affecting a high number of targets in the plant cell [4,5]. Activation of ABA signaling in this ligand-dependent manner leads to plant protection during water deficit (Figure 1). Future applications in agriculture were envisaged when the structural and mechanistic insights into ABA signaling were unveiled. For instance, the recent development of small synthetic molecules acting as ABA-agonists (bind to the ABA receptor and activate ABA signaling) or ABA-antagonists (occupy the receptor and block ABA-mediated responses) offers key tools to modulate ABA signaling and potential use in agriculture [6-8] (Figure 1). Spraying with ABA-agonists could turn on the ABA stress response pathway, which regulates both water loss and root growth, promotes accumulation of compatible solutes and synthesis of protective dehydrins. ABA-antagonists could favor seed germination or might prevent overactivation of ABA signaling in pollen [8]. However, these chemicals have yet to be approved for safe use in agriculture. Alternatively, constitutive or inducible overexpression of improved ABA receptors in transgenic crops might also represent a valuable strategy that does not require the addition of exogenous compounds [9,10].

Sir Frances Bacon published in 1625: "if the hill will not come to you, you must go to the hill". Thus, if current agrochemicals do not positively influence plant response to drought yet, the scientific community must endow them with such new property. In this direction, Park *et al.*, [11] report a new pioneering strategy to trigger the plant ABA response using engineered ABA receptors that are able to accommodate already-in use agrochemicals. These authors started their work with 15 agrochemicals that lack the capability to act as ligands of ABA receptors. Obviously, these chemicals were not designed to this end because ABA receptors show high specificity for their natural ligand. However, after saturation mutagenesis of 25-ABA ligand proximal residues of the PYR1 ABA receptor [9], the authors were able to identify engineered PYR1 receptors that bind weakly to 4 of the 15 compounds tested (Figure 1). Additional rounds involving combinatorial, saturation and shuffling mutagenesis allowed the generation of a hextuple PYR1 mutant, named PYR1^{MANDI}, which possesses nanomolar sensitivity for one agrochemical named mandipropamid (MD) to inhibit *in vitro* the PP2C HYPERSENSITIVE TO ABA (HAB1) (Figure 1). This compound is currently used to control oomycete pathogens, formerly known as pseudo-fungi or water mold, but currently belonging to a different supergroup of the domain Eukarya than Fungi. Among the oomycetes, there are severe plant pathogens, e.g. *Plasmopara viticola* and *Phytophthora infestans*. This latter oomycete caused the great potato blight and famine in Ireland (1845-1850). *P. infestans* is still a serious disease and it is controlled by regular spraying with agrochemicals, such as MD. Since the PYR1^{MANDI} receptor is now able to accommodate the agrochemical, which was also documented to atomic resolution by elucidating X-ray crystal structure of a ternary complex with the mutated receptor and HAB1, a new use has been established for MD, i.e. the activation of ABA signaling in transgenic plants expressing PYR1^{MANDI}. To this end the authors were able to show that MD induces an ABA-like transcriptional response and is able to control transpiration and drought tolerance in *Arabidopsis thaliana* plants overexpressing PYR1^{MANDI}. Therefore, MD qualifies as orthogonal agonist of the ABA pathway since it is silent (with respect to ABA signaling) in normal cells but in transgenic cells expressing the neo-receptor PYR1^{MANDI} triggers activation of ABA signaling.

In tomato (*Solanum lycopersicum*) plants expressing the *35S:PYR1*^{MANDI} transgene, the combination of MD+PYR1^{MANDI} was able to control leaf temperature, suggesting that this approach might be useful to modulate ABA signaling in crops. A potential limitation of this strategy would be that transgenic PYR/PYL ABA receptors were not able to efficiently inhibit crop PP2Cs, since higher specificity for the receptor-phosphatase interaction might be expected when homologous components interact each other. However, it has been shown that tomato PYR/PYL ABA receptors are able to inhibit *Arabidopsis* PP2Cs and confer enhanced drought tolerance when overexpressed in *Arabidopsis* [12]. Thus, it seems that ABA receptors can be functionally exchanged between species. Additionally, crop ABA receptors could be tailored to efficiently bind agrochemicals. Although further experiments are needed in different crop species, it might be expected that this proof of the concept could be efficiently translated into transgenic crops to enhance drought tolerance. Thus, if this dream becomes reality, it could happen that an agrochemical able to prevent severe oomycete-promoted plant diseases gains the ability to confer plant drought tolerance in crops. In the absence of the engineered ABA receptor, the agrochemical only would exert its original function, which avoids undesirable side effects when treating oomycete infections.

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Figure 1. ABA signaling is controlled in a ligand-dependent manner. (a) Dimeric PYR1, a member of the PYR/PYL/RCAR family of ABA receptors, perceives ABA intracellularly and in its monomeric state forms stable ternary complexes with clade A PP2Cs, thereby inactivating them. It allows the phosphorylation and activation of SnRK2s, which in turn phosphorylate different targets in plant cells to mount the ABA response under stress conditions. (b) Different approaches have been followed to identify small molecules able to act as agonists or antagonists of ABA signaling. The structure of a 3'-alkylsulfanyl-ABA with a 6-C extension (AS6), which acts as ABA-antagonist, is shown. Quinabactin is a sulfonamide ABA-agonist. Mandipropamid is a mandelamide orthogonal agonist able to activate an engineered PYR1 receptor ($PYR1^{MANDI}$). Images were generated with the LigandScout software tool using 3NEF, 3QN1, 4LA7 and 4WVO Protein Data Bank codes. (c) An agrochemical can be converted into an ABA orthogonal agonist through the use of engineered ABA receptors. First, the K59R mutation abolishes ABA-binding capacity of PYR1. Second, weak binding of agrochemicals can be detected in receptor allele collections where the ABA-binding pocket has been mutagenized. Finally, combinatorial and shuffling mutagenesis generates an engineered PYR1 receptor that shows high affinity for binding of a certain agrochemical. (d) The ABA signaling pathway can be activated in *Arabidopsis* or tomato plants expressing $PYR1^{MANDI}$ through mandipropamid treatment.

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To the attention of the referees,

We acknowledge very much the different comments to improve the ms. Corrections to address all referee´s suggestions are labeled in red. Additionally, we have introduced some changes concerning oomycetes. Upon discussion with experts in taxonomy, we realized that oomycetes (formerly known as pseudo-fungi or water mold) currently belong to a different supergroup of the domain Eukarya than Fungi. Therefore, although the agrochemical is still sold as “fungicide”, it is not correct to match oomycetes and fungi.

Concerning the comment of reviewer 2, we note that in the absence of the engineered ABA receptor, mandipropamid only would exert its original function, which avoids undesirable side effects when treating oomycete infections in non-transgenic plants. If necessary, other agrochemicals would be available to treat oomycete infection in transgenic plants expressing $PYR1^{MANDI}$.

Sincerely

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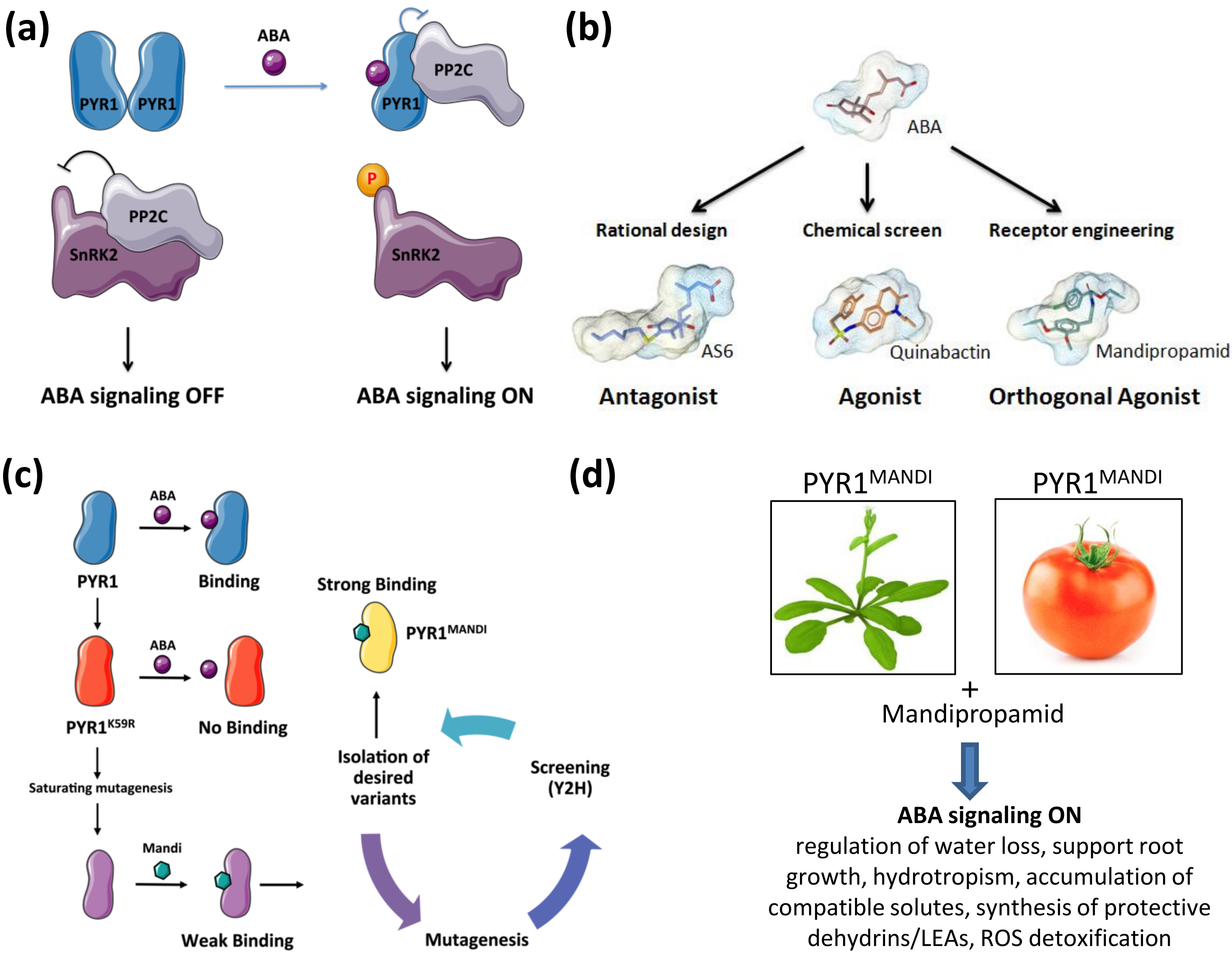
Figure



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