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Unnatural agrochemical ligands for engineered abscisic acid receptors

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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.

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, limited water availability is the single most important factor that reduces crop yield and 70% of global fresh water resources are currently used by agriculture [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, because 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 (binding to the ABA receptor and activating ABA signaling) or ABA-antagonists (occupying the receptor and blocking ABA-mediated responses) offers key tools to modulate ABA signaling with potential application in agriculture [6–8] (Figure 1). Spraying with ABA-agonists could turn on the ABA stress response pathway, which regulates water loss and root growth and 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, the scientific community must endow them with such new property. Towards this goal, Park *et al.*, [11] report a new pioneering strategy to trigger the plant ABA response by using engineered ABA receptors that are able to accommodate already-in use agrochemicals. The authors started their work with 15 agrochemicals that lack the capability to act as ligands of ABA receptors. Obviously, these chemicals were not designed for this purpose, 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

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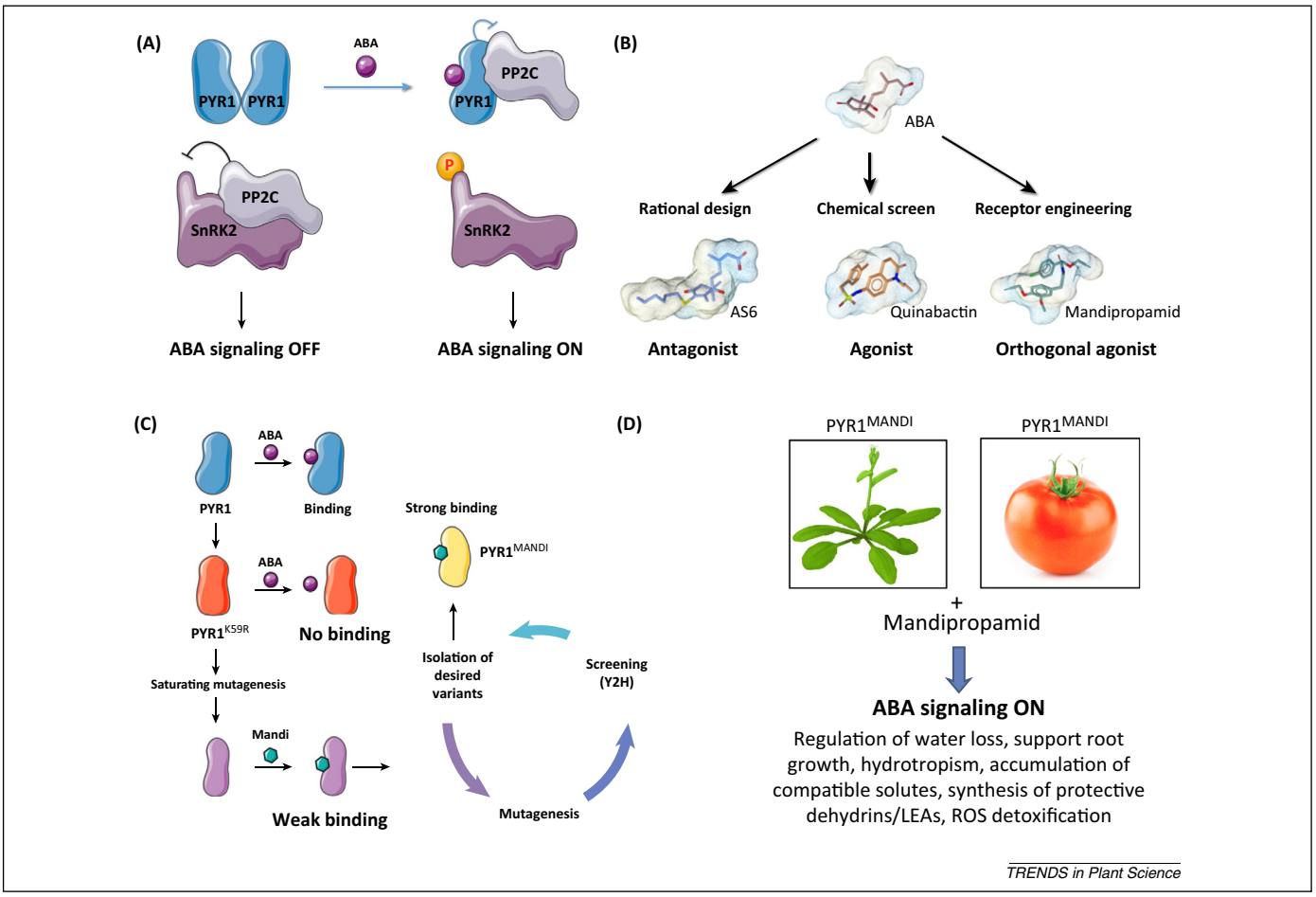

Figure 1. ABA signaling is controlled in a ligand-dependent manner. (A) Dimeric PYR1, a member of the PYR/PYL/RCAR family of ABA receptors, perceives ABA intracellularly and in its monomeric state forms stable ternary complexes with clade A PP2Cs, thereby inactivating them. It allows the phosphorylation 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.

$PYR1^{MANDI}$, 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*. *Phytophthora infestans* caused the great potato blight and famine in Ireland (1845-1850), is still a serious disease and it is controlled by regular spraying with agrochemicals, such as MD. Because the $PYR1^{MANDI}$ receptor is now able to accommodate the agrochemical, which was also documented at 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, because 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, because higher specificity for the receptor-phosphatase interaction might be expected when homologous components interact with 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 would only exert its original function, which avoids undesirable side effects when treating oomycete infections.

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Rhizobial root hair infection requires auxin signaling

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Legumes can enter into a mutualistic relationship with nitrogen-fixing rhizobacteria. A recent study by A. Breakspear *et al.* sheds new light on the mechanisms involved in rhizobial infection of their host root hair during symbiosis establishment and reveals a new role for auxin signaling in this process.

Symbiotic infection in root nodule symbioses

Nitrogen availability is one of the most important factors limiting plant growth worldwide. Only some bacteria have the ability to exploit nitrogen gas (N_2), an abundant nitrogen source, by converting it to ammonia through biological nitrogen fixation. Most plants extract nitrate or ammonium from the soil, but during evolution some plants have acquired the capacity to enter into mutualistic association with nitrogen-fixing bacteria. The most intimate of these associations lead to the formation of new organs called root nodules where the bacteria are hosted in an environment favorable for nitrogen fixation. There are only two groups of plants that can enter nitrogen-fixing root nodule symbioses: legumes and actinorhizal plants. Legumes associate with bacteria collectively referred to as rhizobia, whereas actinorhizal plants form a symbiosis with filamentous actinobacteria of the genus *Frankia*. In most legumes, symbiotic infection occurs through root hairs and the formation of infection threads [1], however how bacterial root hair infection is regulated is not fully understood. In a recent report, Breakspear *et al.* [2] analyzed the 'infectome' that is, the transcriptome of *Medicago truncatula* root hairs infected by *Sinorhizobium meliloti*. In order to identify plant genes specifically involved in rhizobial infection, they analyzed gene expression in root hairs after inoculation by wild-type *S. meliloti* versus a *nodΔD1ABC* mutant unable to produce Nod factors and therefore to infect root hairs. They measured gene expression using microarrays at three timepoints: prior to root hair curling [1 day post inoculation (dpi)], after root hair curling and infection pocket formation, but before infection thread formation (3 dpi) and after

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