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REVIEWS

Concluding Remarks

The intimate interactions of the phyllosphere microbiota with the plant itself determine the plant responses to changing environments and form a regulatory feedback loop that underpins a coevolutionary, mutualistic relationship between the two ecosystem components. Defects in innate immunity to effectively cope with biotic and abiotic stresses are likely a common challenge for the plant kingdom; modulating key host genetic networks to prevent dysbiosis is likely a novel pathway to leverage the native phyllosphere microbiome to improve the natural and agricultural plant performance.

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Spotlight

Drug Discovery for Thirsty Crops

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Following virtual screening and structure-based ligand optimization, researchers have developed opabactin (OP), an abscisic acid (ABA)-receptor agonist with tenfold greater *in vivo* activity than ABA. This new ligand surpasses previous agonists for its potency and bioactivity on staple crops. OP leads a new class of agrochemicals designed to protect crops from drought.

ABA-Like Drugs

Among stresses, drought has the strongest impact on crop productivity and is occurring more frequently and intensely in a changing climate. ABA regulates plant growth and development and is crucial for adaptation to environmental stresses, including drought. In plants, ABA is perceived by the PYR/PYL/RCAR family of ABA receptors that comprises 14 members in arabidopsis (*Arabidopsis thaliana*). PYR/PYL is the largest family of plant hormone receptors and is classified into three different subfamilies: subfamily I (PYL7–10), subfamily II (PYL4–6 and PYL11/12), and subfamily III (PYR1 and PYL1–3). Chemical compounds capable of activating ABA receptors (i.e., ABA-receptor agonists) hold promise for agriculture because their application could reduce yield losses due to drought. Thanks to the abundant crystallographic data gathered on ABA receptors, synthetic ligands found in chemical screenings can now be optimized to efficiently bind and activate PYR/PYL receptors. ABA's coordination in ternary complex with PYR/PYL receptors and PP2C co-receptors involves a network of water-mediated hydrogen bonds and hydrophobic and electrophilic interactions. The 'Trp-lock' stabilizes ternary PYL-ABA-PP2C complexes by a series of hydrogen bonds that engage ABA's ketone with the gate and latch loops of the receptor and a key tryptophan residue of the PP2C (Figure 1A). Additionally, a salt bridge links ABA's carboxylate with a conserved lysine of the receptor (Lys^{59} in PYR1) essential for ABA binding (Figure 1A) [1].

The sulfonamide quinabactin (QB) was the first synthetic ligand able to improve drought tolerance in plants [2]. Mutant analysis indicates that QB activates only the subset of dimeric ABA receptors PYR1, PYL1, and PYL2 *in vivo*. At the structural level, QB and ABA do not look alike (Figure 1B), but QB is able to fit into the receptor's pocket and mimic an important set of hydrophobic interactions and hydrogen bonds established by ABA [2]. Using crystallographic data on QB ternary complexes, several QB derivatives have been developed. AMF4 is a straightforward modification of QB where four fluorine atoms have been appended to the methylphenyl group, increasing the *in vivo* activity of the molecule and its persistence (Figure 1B) [3]. In subsequent work, QB's dihydroquinolinone was replaced by a benzyl ring decorated with a nitrile and a cyclopropyl moiety to generate cyanabactin (CB) (Figure 1B) [4]. CB's cyano group serves as hydrogen acceptor and engages the 'Trp-lock'. CB's cyclopropyl group occupies the 3′tunnel, a hydrophobic cavity that interacts with ABA's 7′ methyl group (Figure 1A) [5]. The interaction with the 3′tunnel increases CB's affinity for dimeric receptors [4].

Similar to the development of sulfonamide-based agonists like QB, the synthesis of ABA structural analogs has been

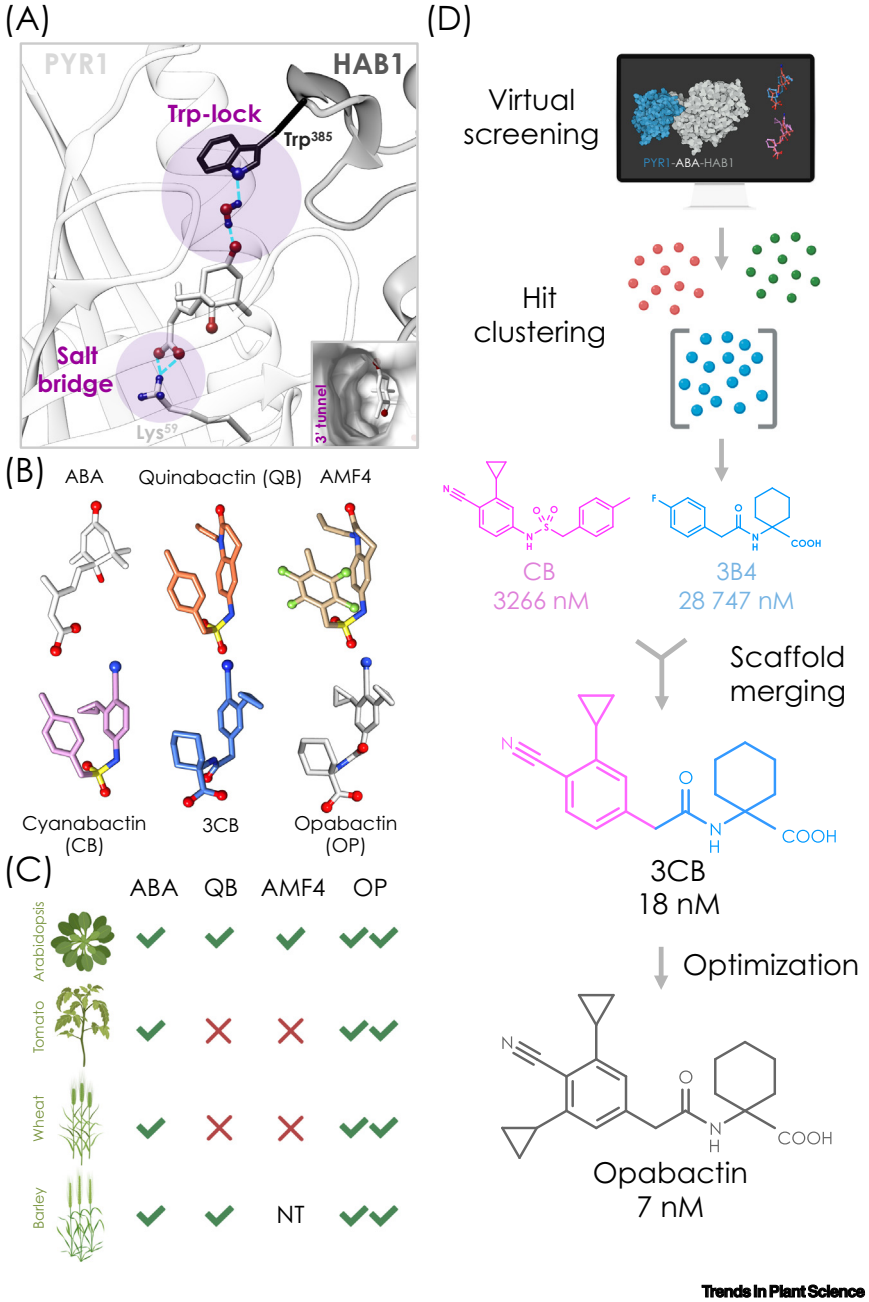

Figure 1. Structure, Design, Activity, and Discovery Process for Opabactin (OP). (A) Structure of the ternary complex PYR1-ABA-HAB1 (PDB: 3QN1) highlighting the 'Trp-lock' and the salt bridge. The 3'tunnel of PYL1 (PDB: 3JRS) is also depicted. (B) 3D structures of different abscisic acid (ABA)-receptor agonists. The 3D structure of OP was obtained by docking using Maestro. (C) *In vivo* activity of ABA, quinabactin (QB), AMF4, and OP in different plant species. The *in vivo* potency of the different compounds is indicated with green ticks or red crosses if the compound was found not active (NT, not tested). (D) Scheme of the discovery process for OP indicating the IC_{50} values of the various molecules obtained through *in vitro* PP2C assays using PYL2 and HAB1 [8]. Some elements of this figure were created using BioRender (https://biorender.com).

instrumental in understanding ABA's structure–activity relationship [6]. Tetralone-ABA (tABA), where the vinyl methyl portion of ABA has been replaced with an aromatic ring, exhibits good *in vitro* activity and is likely to have a longer *in vivo* half-life than ABA because it is unable to cyclize to the catabolite phaseic acid [7].

From the First in Class to the Best in Class

ABA signaling is one of the most interesting targets to improve plant drought tolerance. Dozens of ABA-receptor agonists have been protected with patents. However, Vaidya *et al.* recently found that ABA-like molecules with sulfonamide linkers (e.g., QB, AMF4, and CB) show low activity on important crops like wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*) [8]. To find novel scaffolds to develop potent and broad-spectrum ABA agonists, Vaidya *et al.* performed virtual screening on millions of compounds. Docking experiments were set up to identify ligands that retain interaction with the conserved Lys of the receptor. This constraint helped to identify a set of substituted phenyl-amides whose carboxylate might form a salt bridge with the key Lys of the receptor, overcoming the limitation of the sulfonamide-based ABA agonists described so far. This group of phenyl-amides is specific towards PYL8-like receptors [9]. However, they lack a properly positioned hydrogen acceptor to interact with the 'Trp-lock' water, resulting in low agonist activity on family II and III receptors [9]. The best molecule of this amide group, 3B4 (Figure 1D), shows submicromolar activity towards subfamily I receptors but has poor IC_{50} values on dimeric receptors. To increase the ligand potency, Vaidya *et al.* made use of a medicinal chemistry trick. Playing with the compounds as with LEGO® pieces, they merged the amide of 3B4 with the cyclopropylphenyl group of CB (Figure 1D). Thus, the 3B4's carboxylate provides the key interaction with the conserved Lys, while the cyano group provides the interaction with the Trp-lock water. This scaffold-merging exercise resulted in a chimeric 3B4-CB hybrid,

named 3CB, a synthetic pan-agonist with nanomolar activity for all arabidopsis and wheat receptors tested [8,9].

While 3CB is an exceptional agonist on its own, structural analysis of the PYL10-3CB complex suggested that it could be improved even further. In contrast to CB, 3CB's cyclopropyl group was not oriented towards the 3′tunnel. To optimize 3CB, the authors introduced a second cyclopropyl substituent. In the newly synthesized molecule, the second cyclopropyl is oriented towards the 3′tunnel and improves the activity even further, becoming the most active ABA-receptor agonist described to date. Using slang borrowed from video gamers, the authors called this compound 'opabactin', for 'overpowered ABA receptor activation'. This new compound is an overpowered ligand with ten- and five-times-stronger *in vivo* activity than ABA in germination inhibition and stomatal closure, respectively. Notably, the addition of the second cyclopropyl group in OP reduces its *in vitro* activity on AtPYL8 but increases it sixfold on TaPYL8, despite having stronger *in vivo* activity than 3CB in both plant species.

OP has strong *in vitro* activity over family II and III ABA receptors in both arabidopsis and wheat. However, the exceptional activity of OP on dimeric receptors, five- to tenfold higher than ABA, might be responsible for the 'overpower' of OP. Indeed, genetic analysis in arabidopsis revealed that OP's *in vivo* activity is due to the activation of dimeric PYR1, PYL1, and, especially, PYL2, confirming the relevance of these receptors in seed germination and stomatal closure [2,8,10]. Furthermore, isothermal titration calorimetry (ITC) experiments demonstrated that the binding of OP to ABA receptors is enthalpically driven, a characteristic common to best-in-class drugs [11]. Importantly, OP is able to activate stomatal closure and to reduce transpiration not only in arabidopsis but also in tomato, wheat, and barley (*Hordeum vulgare*) (Figure 1C). After the discovery of QB, the first-in-class ABA-receptor agonist able to improve drought tolerance, OP is currently the best-in-class synthetic antitranspirant.

Still, Challenges Remain

QB was discovered 7 years ago. However, the low activity of QB on staple crops like wheat was not reported until recently [8]. This highlights the importance of extending the characterization of ABA-receptor agonists from arabidopsis to crops or into monocot crop models like *Setaria viridis* or *Brachypodium distachyon*, closely related to staple crops with C4 (maize, sorghum, etc.) or C3 (wheat, rice, etc.) photosynthesis. Efforts in this direction have only recently started to be made [12]. However, data obtained in laboratory setups will need to be confirmed in field trials to fully understand the benefit of antitranspirants under field conditions. We also propose that understanding the chemical and genetic determinants for the bioactivity of these synthetic ligands, in different plant species, will help in the development of the next generation of antitranspirants. Additionally, the combination of synthetic ligands with plants expressing engineered receptors represents another layer of improvement to increase ligand potency and crop productivity while reducing agrochemical input, making this alternative more environmentally friendly [3].

The development of OP, an ABA-receptor agonist with greater potency than the endogenous hormone ABA, is a compelling example of the powerful combination of medicinal chemistry and plant biology and an exceptional advance in our mission to improve plant performance under stress conditions to improve global food security.

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Forum

Long-Lived Trees Are Not Immortal

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Separating out the different effects of ageing on long-lived trees remains challenging. Herein current