Plant Physiology and Biochemistry 227 (2025) 110155

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Natural modulators of abscisic acid Signaling: Insights into polyphenol-based antagonists and their role in ABA receptor regulation

Javier Merino [a,1], María Rivera-Moreno [a,1], Mar Bono [b], Diego Núñez-Villanueva [c], Ana González-Vega [a], Cristian Mayordomo [b], Lourdes Infantes [a], Rupesh Chikhale [d], Pedro L. Rodríguez [b,*], Armando Albert [a,**]

[a] Instituto de Química-Física "Blas Cabrera" (IQF) Consejo Superior de Investigaciones Científicas, ES-28006, Madrid, Spain
[b] Instituto de Biología Molecular y Celular de Plantas (IBMCP) Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia, ES-46022, Valencia, Spain
[c] Instituto de Química-Médica (IQM) Consejo Superior de Investigaciones Científicas, ES-28006, Madrid, Spain
[d] Cambridge Crystallographic Data Centre (CCDC) CB2 1EZ, Cambridge, United Kingdom

ARTICLE INFO

Keywords:
Abscisic acid
Abiotic stress
ABA receptor
Antagonist
Hydroxicinnamic acids
Protein structure
Structural biology

ABSTRACT

The phytohormone abscisic acid (ABA) plays a pivotal role in regulating essential plant processes, including seed dormancy, germination, and stress responses. ABA signaling is mediated by PYR/PYL/RCAR receptors, which interact with clade A PP2C phosphatases to control downstream signaling pathways. Advances in structural biology have enabled the development of synthetic ABA modulators, such as agonists and antagonists, which can enhance or inhibit ABA signaling for agricultural applications. However, the high production costs and potential toxicity of synthetic modulators have motivated the search for natural alternatives. Here, we explore the potential of polyphenols, a class of plant secondary metabolites, as eco-friendly non-canonical ligands for ABA receptors. Through virtual screening and structural analysis, we identified coumaric acid and other hydroxycinnamic acids as natural ABA antagonists. These compounds compete with ABA for receptor binding, disrupting the ABA-dependent inhibition of PP2C phosphatases by PYR/PYL proteins. As a result, they counteract ABA-imposed stress responses in *Arabidopsis thaliana*, promoting seed germination and seedling establishment. Further chemical optimization yielded improved ABA antagonists based on hydroxycinnamic acid and natural amino acid conjugates. Their use in plants provides a sustainable alternative to synthetic modulators and opens new biotechnological strategies for crop management. In addition, our findings highlight a mechanism for fine-tuning ABA receptor activity in vivo through the interaction of ABA receptors with endogenous hydroxycinnamic acids.

1. Introduction

The phytohormone abscisic acid (ABA) regulates essential physiological processes in plants, including seed development, germination, dormancy, and stress responses (Rodriguez et al., 2019). At low ABA concentrations, the subgroup III of SnRK2 kinases forms self-inhibitory complexes with clade A protein phosphatases (PP2Cs) (Soon et al., 2012). When ABA levels increase, the PYR/PYL/RCAR (PYR/PYL) receptor family is activated, leading to the formation of ternary complexes with ABA and the clade A PP2Cs, which is a key step for ABA signaling (Daniel-Mozo and Albert, 2021; Fujii et al., 2009; Ma et al., 2009; Melcher et al., 2010b; Miyazono et al., 2009; Moreno-Alvero et al., 2017; Nishimura et al., 2009; Park et al., 2009; Santiago et al., 2009a). As a result, PP2Cs are inhibited and SnRK2 kinases are released from their inhibitory interaction with PP2Cs. SnRK2s that were previously dephosphorylated by PP2Cs need to be phosphorylated by the upstream activating B2- and B3-type RAF kinases (Lin et al., 2020, 2021; Soma et al., 2023; Takahashi et al., 2020). Subsequently ABA-activated SnRK2s phosphorylate downstream targets, including transcription factors, to promote the expression of ABA-responsive genes (Furihata

* Corresponding author.
** Corresponding author.
E-mail addresses: prodriguez@ibmcp.upv.es (P.L. Rodríguez), xalbert@iqf.csic.es (A. Albert).
[1] Javier Merino and María Rivera-Moreno contributed equally to this work.

https://doi.org/10.1016/j.plaphy.2025.110155
Received 24 March 2025; Received in revised form 23 May 2025; Accepted 10 June 2025
Available online 16 June 2025

et al., 2006; Peirats-Llobet et al., 2016) and various transporters and channels at the plasma membrane, which facilitates stomatal closure to reduce water transpiration at leaves during stress conditions (Ma et al., 2009; Park et al., 2009; Santiago et al., 2009a).

PYR/PYL receptors form a multigenic family of proteins in plants (Rodriguez et al., 2019). They exist in different oligomeric states, namely as monomers or dimers (Dupeux et al., 2011). These structural forms influence their ABA binding affinity and functional roles in ABA signaling. Dimeric ABA receptors have a lower affinity for ABA compared to their monomeric counterparts which, consequently, are more efficient in forming ternary complexes with PP2Cs. This variability enables precise ABA responsiveness and regulation of ABA signaling across a range of fluctuating ABA levels. In addition, it enables specialized roles for PYR/PYLs. For instance, dimeric PYR/PYL receptors are required for processes that require slower or sustained ABA responses, such as seed dormancy or long-term stress adaptation (Wang et al., 2024). However, dimeric receptors also play a fundamental role for regulation of stomatal closure (Okamoto et al., 2013). On the other hand, certain monomeric receptors exhibit lower dependence on high ABA levels for inhibition of PP2Cs, thus providing a baseline level of signaling regulation at basal ABA levels (Dupeux et al., 2011; Hao et al., 2011).

At the molecular level, ABA-mediated receptor activation and subsequent PP2C inhibition require the dissociation of dimeric receptors, along with a structural rearrangement of two key loops, the gate and latch loops, located at the entrance of the binding pocket (Melcher et al., 2009). The latch loop adjusts its structure to accommodate ABA, while the gate loop undergoes further reorganization or closure upon interaction with the phosphatase, facilitating the inhibition of phosphatase activity (Moreno-Alvero et al., 2017). Within the binding pocket, ABA is stabilized by many hydrophobic and water-mediated hydrogen bonds (Fujii et al., 2009; Miyazono et al., 2009; Nishimura et al., 2009; Park et al., 2009; Santiago et al., 2009a). Among them, the hydrogen bond between ABA carboxylate group and a conserved Lys residue, along with a water-mediated interaction linking the ABA ketone group to the latch and gate loops are essential. When in complex with the phosphatase, this water molecule additionally forms a hydrogen bond with a conserved tryptophan residue in the PP2C, establishing a structure known as the "Trp Lock" (Fig. 1A) (Melcher et al., 2009).

Advances in structural studies of protein components involved in ABA signaling have enabled the development of synthetic ABA receptor agonists and antagonists, utilizing techniques originally established for

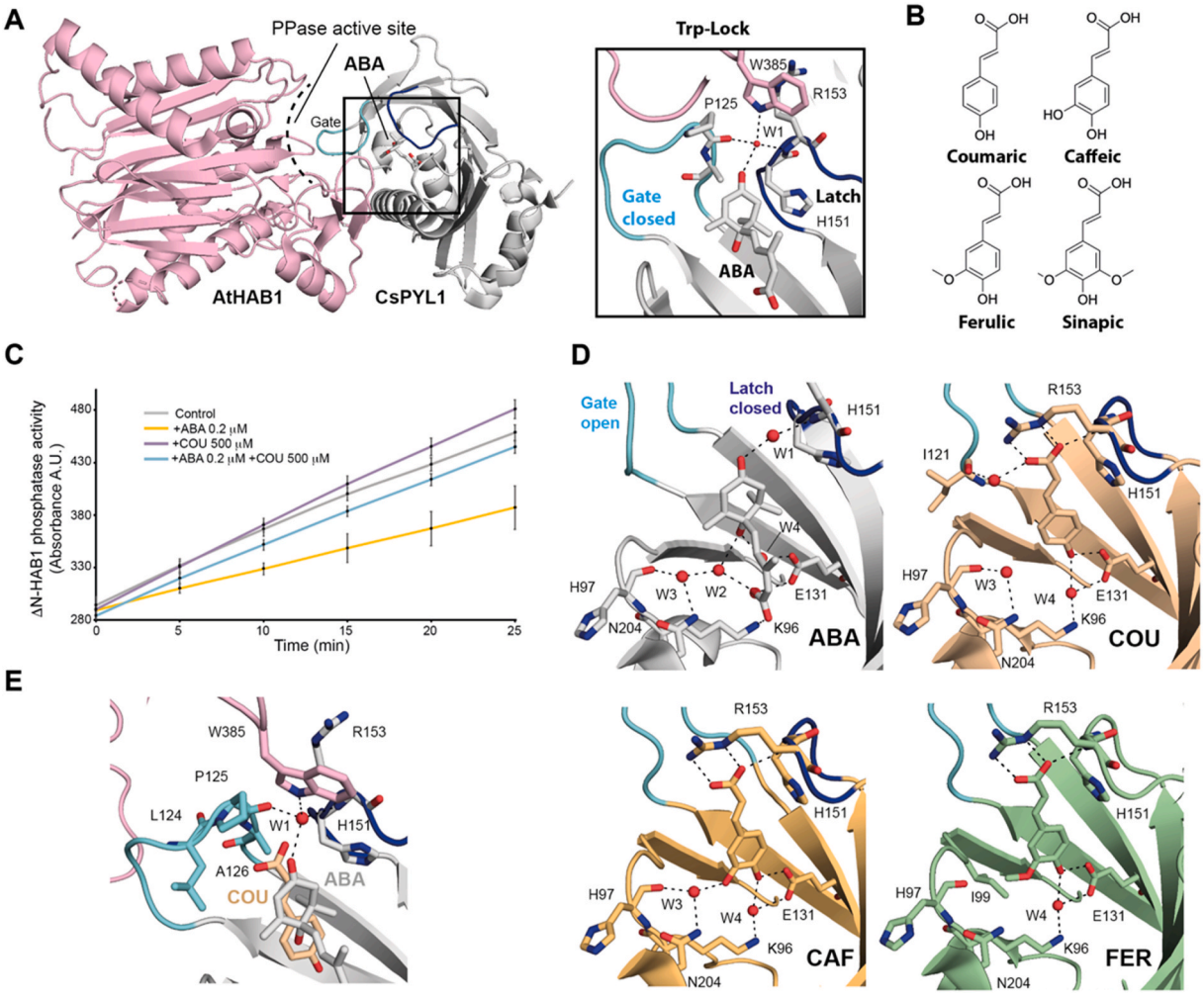


Fig. 1. The structural basis of the hydroxycinnamic acids as modulators of PYR/PYL activity. **(A)** The ternary complex AtHAB1-ABA-CsPYL1, together with a section showing details of the "Trp-lock" feature that links ABA, the gate and latch loops from CsPYL1 and W385 from AtHAB1 (PDB code 5MN0). (B) The chemical structures of the hydroxycinnamic acids. (C) The comparison of the HAB1 activity monitored using *para*-nitrophenylphosphate as a substrate in the presence of CsPYL1, with 500microM COU, 0.250microM ABA and 500microM COU plus 0.250microM ABA. (D) A detailed view of the ABA-binding pocket in SlPYL1 structures in complex with ABA (PDB code 5MNB), Coumaric (COU), Caffeic (CAF) and Ferulic (FER) Acids. Water mediated hydrogen bonds and those between ligands and receptors are represented as dashed lines. All the panels are oriented consistently for comparison (E) A section showing the superimposition of COU (wheat) from its complex with SlPYL1 onto the CsPYL1-ABA-HAB1 complex (PDB code 5MN0).

structure-based drug design (Lozano-Juste et al., 2020). The agonists, have shown potential as agronomic tools to mitigate drought stress, as they enhance plant water-use efficiency by promoting stomatal closure, reducing water loss through transpiration (Cao et al., 2013; Lozano-Juste et al., 2023; Okamoto et al., 2013; Vaidya et al., 2019). Additionally, ABA agonists activate stress-responsive pathways that help plants conserve resources and maintain cellular function under water-limiting conditions, effectively increasing resilience against drought. Conversely, ABA antagonists enhance transpiration (Takeuchi et al., 2014, 2018; Vaidya et al., 2021; Wang et al., 2024). This effect can be beneficial in environments where water is not limiting, as it allows for greater carbon dioxide uptake, improving growth and productivity (Miao et al., 2018; Pizzio et al., 2024). Additionally, ABA antagonists can aid in controlling germination timing, helping to stimulate seed growth even in conditions where ABA would typically inhibit it, which can be valuable in optimizing crop cycles and enhancing yields (Eckhardt et al., 2024; Takeuchi et al., 2014). While agonist molecules mimic the natural interaction pattern of ABA with its receptors, known antagonists employ various mechanisms to impede PP2C inhibition. They may lock the latch in a non-productive conformation (Melcher et al., 2010a), prevent gate closure (Vaidya et al., 2021), block the interaction with the phosphatase (Takeuchi et al., 2014), or interfere with the dissociation of dimeric members of the PYR/PYL family, which is essential for receptor activation (Wang et al., 2024). Despite this knowledge, the widespread application of synthetic modulators of ABA signaling in agriculture is limited due to the high production costs and the need for thorough toxicity testing. Thus, the investigation of naturally occurring plant compounds that might interact with PYL/PYR receptors, offers a sustainable alternative. However, few examples have been reported in contrast to the myriad of synthetic molecules (Hewage et al., 2020; Infantes et al., 2022; Wang et al., 2024).

We define non-canonical natural ligands of ABA receptors as naturally occurring compounds that may differ structurally from ABA but can still bind to and activate or inhibit ABA receptors to some extent. These ligands are present in plants or their associated organisms and may contribute to fine-tuning ABA receptor activity under specific environmental or physiological conditions. Examples include phaseic acid, a catabolite of ABA, which can act as an agonist of certain ABA receptors (Weng et al., 2016) or niacin, the vitamin B3, which functions as a weak antagonist of certain PYR/PYL receptors in the μM range (Infantes et al., 2022). However, given that niacin is a precursor of nicotinamide adenine dinucleotide (NAD^+), its typical plant concentrations are in the millimolar to sub-millimolar range (Zhang and Mou, 2009) and it might function as a physiological modulator of ABA activity.

Polyphenols are a major class of bioactive phytochemicals, classified as secondary metabolites, that are abundant in a wide variety of plant-based foods (Rodriguez-Larrea et al., 2010; Sikuten et al., 2020). They are well-known for their role in enhancing plant resilience against pests and environmental stresses (Erb and Kliebenstein, 2020; Zagoskina et al., 2023). This prompted us to explore whether polyphenols could act as non-canonical ligands of ABA receptors and/or can be repurposed as eco-friendly ABA response modulators. Virtual screening of large chemical libraries, an *in silico* method to predict ligand-receptor interaction, has proven effective in identifying compounds that mimic ABA action (Lozano-Juste et al., 2023). Using this approach, we screened the Phenol-Explorer database, a comprehensive resource on polyphenol compounds (Rothwell et al., 2013). Through this screening, followed by crystal structure determination, we identified coumaric acid and its derivatives as potential eco-friendly ABA antagonists. We showed that they can restore PP2C activity in the presence of ABA and PYR/PYL dimeric receptors and, in addition, we demonstrated that these molecules restore seed germination and establishment in *Arabidopsis thaliana* under ABA-imposed stress. Moreover, an additional step of chemical optimization led to an improved class of ABA antagonists, based on the conjugation of hydroxycinnamic acids with natural aromatic amino acids. Our findings suggest that these compounds represent a family of non-canonical natural ligands for ABA receptors, offering a unique mechanism to fine-tune receptor activity under specific environmental or physiological conditions.

2. Materials and methods

2.1. Virtual screening

The molecular models for the virtual screen were prepared from the SMILES, available at the database site (http://phenol-explorer.eu/) (Rothwell et al., 2013) minimized with the Cambridge Crystallographic Data Center (CCDC)'s conformer-generator program and stored as.mol2 files.

The virtual databases were screened using CCDC's Genetic Optimization of Ligand Docking (GOLD) software (Jones et al., 1997). The coordinates of the proteins CsPYL1 and SlPYL1 (PDB codes 5MN0 and 5MOB) were prepared following the standard software's procedure, from previously determined crystal structures (Moreno-Alvero et al., 2017) to account for gate close and gate open conformations of ABA receptors. All water molecules except for those directly involved in ligand binding, and the bound ligand were removed, whereas hydrogen atoms were modelled. The natural ligand abscisic acid (ABA) was used to define the binding site, and it was extended by 7 Å around it. A docking protocol was designed using the software's descriptors to account for the most important interactions that occur in the ABA binding site, including the hydrogen bond to the water lock, the polar network with water molecules deep in the binding pocket and the hydrophobic belt (Moreno-Alvero et al., 2017). These descriptors contributed to the chemPLP scoring function to rank the molecules accordingly (Korb et al., 2009). This ranking was used for an initial filtering, followed by a visual inspection to allow the identification of a final list of candidate compounds.

2.2. Protein preparations

The ABA receptors used in this study were: sweet orange (*Citrus sinensis)* CsPYL1 and tomato (*Solanum lycopersicum)* SlPYL1. The protein phosphatase co-receptor was the ΔNHAB1 from *A. thaliana*. All the proteins were produced following slight modifications of a standard procedure, as described before (Bono et al., 2024; Infantes et al., 2022; Moreno-Alvero et al., 2017; Santiago et al., 2009b). In short, the cDNAs were cloned into the pETM11 expression vector and expressed in *E. coli* (DE3). The proteins were purified by standard immobilized metal affinity chromatography (IMAC). The purification process was monitored by SDS-PAGE and Coomassie blue staining. The proteins concentrations were measured spectroscopically. Each of the proteins were treated in their own buffers: for CsPYL, 30 mM Tris pH 8.0 and 5 mM β-mercaptoethanol; for SlPYL1, 30 mM Tris pH 7.5, 150 mM NaCl and 1 mM dithiothreitol (DTT; For ΔN-AtHAB1, 30 mM Tris pH 7.5, 150 mM NaCl, 1 mM MnCl, 5 % (v/v) GlyOH and 1 mM DTT.

2.3. Phosphatase inhibition assays

The biological effect of the selected hydroxycinnamic acids was assessed by a phosphatase activity assay, which takes advantage of a phosphatase inhibition induced by the PYR/PYL receptors upon ABA binding (Lozano-Juste et al., 2021) The ΔN-AtHAB1 phosphatase activity was monitored using 25 mM *para*-nitrophenylphosphate (pNPP) as a substrate. The reactions were performed in quadruplicates in 25 mM Tris-HCl, pH 7.5 buffer containing 150 mM NaCl, 1 mM dithiotreitol (DTT) and 1 mM $MnCl_2$. Protein concentrations were adjusted to a 4:1 receptor-to-phosphatase molar ratio (1 μM PYR/PYL, 0.25 μM ΔN-AtHAB1), and the indicated concentrations of several ligands, in the presence of 10 % (v/v) DMSO. The reaction was incubated for 10 min at room temperature, prior to the addition of the pNPP. In addition, the

antagonist effect was measured by exposing the ligands to the IC50 concentration of ABA for each protein, which produces a ≈50 % inhibition of the phosphatase, in order to detect the recovery of the phosphatase activity upon ligand-induced displacement of ABA. The assay was performed at room temperature and it was monitored in a BioRad Model 3550 UV-plate reader, measuring the *para*-nitrophenol absorbance at 405 nm every 5 min over a 30 min time range. The activity was normalized by calculating the slope after 30 min, considering as 100 % phosphatase activity the result corresponding to the HAB1:CsPYL complex in the absence of any ligand.

2.4. Binding affinities

The binding affinities of the hydroxycinnamic acids and derivative molecules to the ABA receptors were measured using a Nanotemper Monolith X MST instrument, following the standard manufacturer's procedures. The ABA receptors were fluorescently labelled in the His-tag using the *RED-tris-NTA 2nd Generation*. Briefly, the purified receptors were buffer-exchanged to the assay buffer (50 mM HEPES pH 7.5 supplemented with 0.05 % (v/v) Tween 20, 5 % (v/v) DMSO and 0.5 mM β-mercaptoethanol) and kept at 200 nM. Then, it was mixed at a molar ratio 2:1 protein to dye and incubated for 30 min at room temperature while covered from the light. Labelled proteins were stored frozen as ready-to-use aliquots. Binding curves were prepared by two-fold serial dilutions of the ligands. From a solution at high ligand concentration (between 5 and 20 mM), up to 16 dilutions were mixed 1:1 with the labelled protein and incubated at room temperature for 10 min, then spun at 10000×*g* for 10 min. Protein-ligand solutions were loaded in the capillaries and measured at 80 % beam power. Microscale thermophoresis (MST) was measured at a medium IR laser power setting. Binding curves were obtained either from the MST or from the spectral shift data, as indicated and the data points were fitted to a standard Kd binding model. Data analyses were performed in the manufacturer's MO.Affinity Analysis software.

2.5. Crystallization, diffraction data collection and structure solution and refinement

SlPYL1 was purified to homogeneity by size exclusion chromatography (SEC) for crystallization. It was subsequently concentrated to 10 mg/mL and set for crystallization as described previously (Moreno-Alvero et al., 2017). For the ligand-bound structures, the compounds dissolved in 100 % (v/v) DMSO were added to the concentrated protein prior to setting the crystallization drop, to a concentration of 4 mM and a maximum of 2 % (v/v) dimethyl sulfoxide DMSO. Crystals grew in 2 M ammonium sulphate and pH 7.0 after 72 h. They were cryoprotected with the same precipitation solution supplemented with 30 % (v/v) glycerol and flash-frozen in liquid nitrogen.

Diffraction data were collected at ALBA synchrotron (BL13 beamline), processed with AutoProc using the extended anisotropic method (Vonrhein et al., 2018) and merged with AIMLESS from the CCP4 package (Agirre et al., 2023; Martinez-Ripoll and Albert, 2023). The crystal structures of SlPYL1 in its apo form (PDB code 5MOA) and SlPYL1 in complex with ABA (5PDB code MOB) were used to phase the diffraction data. Final models were obtained after several cycles of restrained refinement with PHENIX (Adams et al., 2010) and manual model building with COOT (Emsley and Cowtan, 2004). Details on data processing and refinement are shown in Table S1 Structural figures were prepared using PyMOL (DeLano and Schrödinger, 2002) and Moorhen (https://moorhen.org/).

2.6. Synthesis of hydroxycinnamic acids derivatives

The synthesis of phenylalanine and tyrosine hydroxycinnamic acid derivatives was carried out using established solution-phase organic synthesis protocols, involving a two-step procedure: a coupling reaction between the hydroxycinnamic acids and ester-derived amino acids, followed by ester cleavage. Detailed experimental procedures, including full characterization data, ^{1}H and ^{13}C NMR spectra, and HPLC chromatograms for all synthesized compounds, are provided in the Supporting Information. For the coupling step, triethylamine (1.2 eq.) was added to a solution of H-Phe-OMe·HCl (1.2 eq) or H-Tyr-O^{t}Bu (1.2 eq.) and the corresponding hydroxycinnamic acid (1 eq.) in anhydrous CH_2Cl_2 or CH_2Cl_2/DMF (10:1) at 0 °C and under argon atmosphere. Under a stream of argon, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.2 eq.) was added and the resulting solution was stirred at room temperature for 3 h. The solvent was removed under vacuum and the crude was then dissolved in ethyl acetate and washed with 1M HCl aqueous solution (2×) and brine (1×). The organic layer was dried over $MgSO_4$ and concentrated under vacuum. In some cases (see Supporting Information), the residue was used without further purification. Otherwise, purification was performed using flash column chromatography. In the second step, the ester derivatives were hydrolyzed to yield the corresponding carboxylic acid products. Phenylalanine derivatives were treated with lithium hydroxide (3 eq.) in THF/water for 2 h. The reaction was diluted with ethyl acetate and washed with 1M HCl aqueous solution (2×) and brine (1×). The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash column chromatography to afford COU-PHE (36 % overall yield), CAF-PHE (29 % overall yield), FER-PHE (74 % overall yield) and SIN-PHE (50 % overall yield). For tyrosine derivatives, the *tert*-butyl ester derivatives were dissolved in CH_2Cl_2/trifluoroacetic acid (1:1). After 3 h of stirring at room temperature, the solvent was evaporated to dryness and the residue was purified by reverse-phase flash column chromatography to afford CAF-TYR (37 % overall yield) and FER-TYR (48 % overall yield).

2.7. Cotyledon growth and root length assays

Approximately 25 seeds (two replicates per experiment) of Arabidopsis thaliana Col-0 seeds were sown on 24-well plates containing MS medium, which was mock- or ligand-supplemented at the indicated concentration. Seeds were stratified in the dark at 4 °C for 3 days, and the plates were transferred to a growth chamber (Sanyo MLR-351) set at 23 °C with a 16 h light/8 h dark photoperiod. Cotyledon growth was scored as the cotyledon area from seedlings that developed green, expanded cotyledons and the first pair of true leaves at 10 days after sowing (das). Growth assays in the absence (mock) or presence of 0.25 μM ABA alone or combined with 50 or 100 μM hydroxycinnamic acids or conjugated molecules were performed in 24-well plates. Each well contained a different treatment, and the experiment was repeated at least twice. In addition to the cotyledon area, the root length of established seedlings was measured at 10 das. Image files suitable for quantitative analysis of cotyledon area or root length were analyzed using the NIH Image software ImageJ.

2.8. Statistical analysis

Statistical comparisons between mock and treated samples were performed by either pairwise t-tests or One-way ANOVA using GraphPad Prism 9. The values for N, P, and the specific statistical test performed for each experiment are included in the appropriate figure legend or main text.

3. Results

3.1. Identification of hydroxycinnamic acids as ABA receptor antagonists through virtual screening and structure-guided optimization

We performed a virtual screening using the GOLD from Cambridge Crystallographic Data Centre (Jones et al., 1997) to identify potential ABA mimics from the molecules of the Phenol-Explorer database (http

://phenol-explorer.eu/) (Rothwell et al., 2013) using the structure of the ternary complex comprising *Citrus sinensis* (sweet orange) PYL1 receptor (CsPYL1), *Arabidopsis thaliana* HAB1 PP2C phosphatase (AtHAB1) and ABA (Moreno-Alvero et al., 2017) as target (Fig. 1A). The structures of CsPYL1 and SlPYL1 were selected for virtual screening and analysis as they were previously solved by our group in complex with *Arabidopsis* HAB1, and we have extensive experience working with these proteins in crystallographic studies (Moreno-Alvero et al., 2017). The PYL1 receptor architecture is highly conserved across plant species, particularly at the ABA-binding and PP2C-interacting interfaces, making these models suitable for structure-based design of ligands with cross-species activity. Notably, synthetic agonists designed using these receptors have demonstrated activity in *Arabidopsis*, tomato, and wheat (Lozano-Juste et al., 2023). After docking, we visually screened the top-scoring hits based on their potential activity, using descriptors that evaluate the presence of acceptor groups near the ketone and carboxylate groups of ABA. This process led to the identification of p-coumaric acid (COU) as a potential hit (Fig. 1B). COU and other hydroxycinnamic acids are phenolic compounds found in many plants, including fruits, vegetables, and grains (Sikuten et al., 2020). They play various roles in plant growth, structural integrity, and defense mechanisms and they are central constituents of defense polymers such as lignin and suberin (Canto-Pastor et al., 2024; Erb and Kliebenstein, 2020; Rodriguez-Larrea et al., 2010; Serra and Geldner, 2022; Zagoskina et al., 2023). Hence, we decided to test its ability to inhibit the AtHAB1 in the presence of CsPYL1 or to restore phosphatase activity in the presence of ABA and CsPYL1 (Fig. 1C). Our data indicate that while 500 μM COU does not affect significantly AtHAB1 activity in the presence of CsPYL1, the molecule exhibits antagonist activity as it restores the decrease in phosphatase activity induced by 0.2 μM ABA, a value close to the dissociation constant (Kd) of ABA in the ternary complex with CsPYL1 and AtHAB1 (Lozano-Juste et al., 2023).

To understand the molecular basis of the mechanism of action of COU and to establish potential rules that might help to find natural active derivatives, we solved the crystal structure of *Solanum lycopersicum* (tomato) PYL1 receptor (SlPYL1) in complex with COU. The electron density map clearly identifies a COU molecule within the ABA binding pocket (Fig. S1). Our data revealed that the structure of SlPYL1 in complex with COU shows a dimeric "latch-closed, gate-open" conformation, similar to the structure observed in the ABA-bound SlPYL1 complex (Moreno-Alvero et al., 2017) (Fig. 1D). The hydroxyl group of COU forms a hydrogen bond with Glu 131 and is connected to the conserved Lys 96 via hydrogen bonds through a water molecule (W4). Meanwhile, the hydrophobic phenyl ring of COU occupies a position comparable to the cyclohexenone group of ABA. Additionally, the carboxylate group of COU protrudes form the position of ABA's keto replacing the water molecule (W1) involved in the Trp-Lock.

The guanidinium group of Arg 153 further stabilizes the ligand within the binding pocket by the formation of a salt bridge with the carboxylate group of the COU. Superimposing this structure with the AtHAB1:ABA:CsPYL1 complex (Moreno-Alvero et al., 2017) suggests that Arg 153 and COU's conformation could prevent gate closure due to steric hindrance with the conserved Leu-Pro-Ala residues at the gate loop, thereby supporting its role as an antagonist (Fig. 1E).

In an attempt to discover natural derivatives of COU that also might display antagonist activity, we paid attention to the joint analysis of the structures of SlPYL1 in complex with COU and ABA (Fig. 1D). Replacing water molecules in the binding pocket is important in structure-based medicinal chemistry projects because it can modulate the binding affinity, specificity, and pharmacological properties of a ligand (Barros et al., 2023). In the ABA binding pocket, such modulation might occur by replacing water molecules (W2 or W4) that form hydrogen bonds with the hydroxyl group and carboxylate of ABA (Fig. 1D). Thus, we envisioned that natural derivatives of COU with additional hydroxyl or methoxy substitutions at the meta position could enhance their affinity for SlPYL1.

To test this hypothesis, we analyzed the antagonist activity of ferulic (FER), caffeic (CAF) and sinapic (SIN) acids (Fig. 2A). Our data indicate that a double substitution at the meta position hinders antagonist activity (SIN), whereas a single substitution conserves it (FER and CAF) (Fig. 1B). To quantitatively estimate the relative affinities of hydroxycinnamic acids compared to ABA, we measured their dissociation constants (Kd) for CsPYL1 using Spectral Shift Analysis to monitor ligand binding (Fig. 2B and Fig. S2). These data confirmed that COU, CAF and FER bind to PYR/PYL receptors and showed that FER and COU display higher affinities for CsPYL1 compared to CAF, with their affinities being 5- to 8-fold lower than that of ABA. We did not observe SIN binding to CsPYL1 in the experimental conditions tested.

To reveal the basis for the observed activities, we solved the crystal structures of the SlPYL1 complex with FER and CAF (Fig. 1D and Fig. S1). The structural data showed that the oxygen atom in the meta position of the aromatic ring on both CAF and FER mimics the position of the aforementioned water molecule (W2) observed in the ABA complex. Notably, the larger methoxy group in FER adapts well within the binding pocket, replacing an additional water molecule (W3), forming hydrophobic interactions with Ile 99 side chain and a CH..O hydrogen bond connecting the methyl group of FER and the backbone carbonyl of His 97. This will explain the enhanced affinity of FER with respect to that observed for COU or CAF.

3.2. Hydroxycinnamic acids block ABA signaling in vivo

To evaluate whether hydroxycinnamic acids can counteract the effects of ABA in vivo, we conducted seedling establishment assays in *Arabidopsis thaliana*. ABA is known to inhibit the seedling establishment and early growth, so we investigated whether coapplying hydroxycinnamic acids at varying concentrations with ABA could alleviate this inhibition. As shown in Fig. 2C and D, 50 μM concentration of COU, CAF and FER effectively mitigated the ABA-induced inhibition of seedling establishment and early growth, with significant relief observed at ABA concentrations of 0.25 μM ABA. These findings identify hydroxycinnamic acids as effective modulators of ABA activity in vivo.

3.3. Scaffold merging of hydroxycinnamic acids and natural aromatic amino acids to produce a class of ABA antagonists that block gate closure

Our structural analysis shows that the carboxylate group of hydroxycinnamic acids hinders receptor activation by preventing gate closure and subsequent interaction with PP2Cs. This suggests that derivatives conjugated at the carboxylate position may further stabilize the receptor in an inactive, gate-open conformation, effectively blocking PP2C activity. To explore this further, we searched the Phenol Explorer database for hydroxycinnamic acid derivatives, which are mainly esters or amino acid conjugates linked through the carboxyl group to natural components such as sugars or natural amino derivatives. Among these, coumaroyl tyrosine (COU-TYR) emerged as a candidate based on virtual screening. Hence, we hypothesized that a scaffold merging approach combining hydroxycinnamic acid headgroups with an aromatic Phe or Tyr amino acid linked via a natural amide scaffold, could preserve binding affinity (Fig. 3A). This design aims to enable simultaneous interactions with the ABA binding pocket and stabilize the gate in an open conformation, as observed with PYL2 and PAnE (Takeuchi et al., 2018) and PYL1 ANT (Vaidya et al., 2021). To evaluate this, we synthesized and characterized the chimeric compounds and tested their activity on CsPYL1 and HAB1 (Fig. S2).

We first measured the Kd of the conjugated molecules. Our data indicate that all conjugate molecules tested display larger affinity than their respective hydroxycinnamic acids derivatives (Fig. 3B and C). Remarkably, COU-Phe and FER-Phe display Kd values for CsPYL1 of 0.153 mM and 0.063 mM, which are comparable to that of ABA, i.e.,0.120 mM. According to that, the analysis of the antagonist activity of these compounds revealed that inhibition of the HAB1 phosphatase

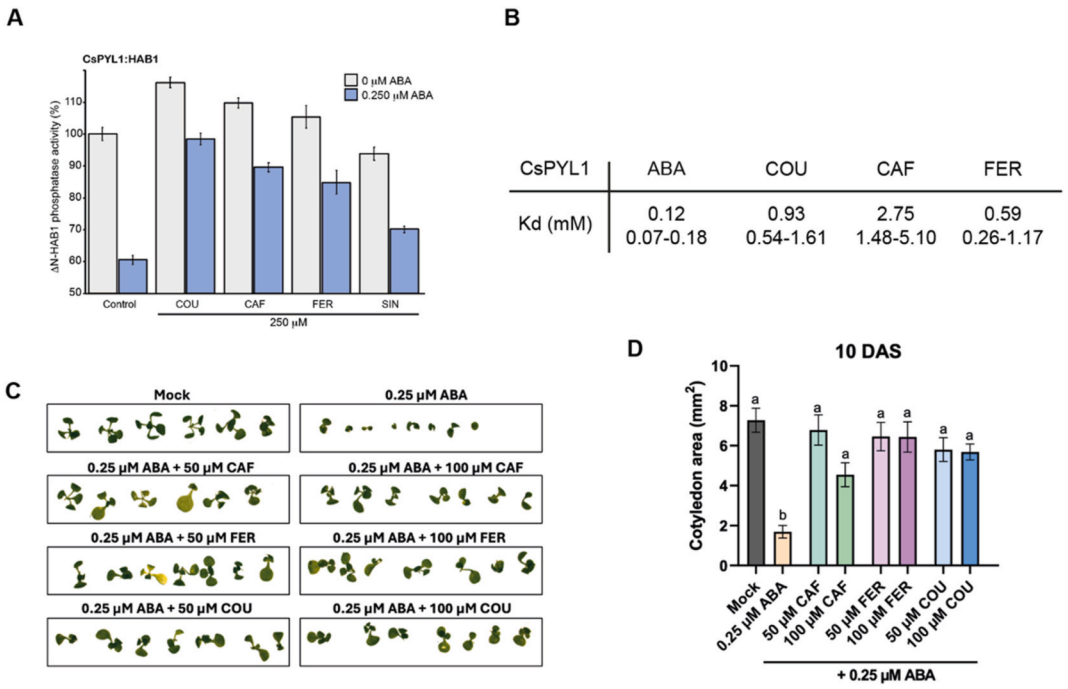


Fig. 2. Hydroxycinnamic acids bind CsPYL1 and display ABA antagonist activity in vitro and in vivo. **(A)** Antagonist effect or hydroxycinnamic acids on AtHAB1 activity through a phosphatase assay. CsPYL-AtHAB1 were incubated with or without 0.25 μ M ABA with the indicated ligands at 500μ M. The data are presented as the mean ± standard deviation. **(B)** The K_d (mM) values of ABA and hydroxycinnamic acids for CsPYL1 as determined using fluorescence spectral shift using the Monolith X (NanoTemper Technologies) and performed in duplicates. The confidence values are indicated below the K_d. **(C)** Hydroxycinnamic acids restore cotyledon growth in seedlings germinated in the presence of 0.25 μ M ABA. Cotyledon growth was scored at 10 d after sowing. **(D)** Quantification of cotyledon area in the experiment described in (C). Bars indicate the mean of 20 cotyledon areas from two independent experiments (N = 10 cotyledons per well from each experiment quantified) ± SE. Cotyledon area was measured in the absence (mock) or presence of 0.25 μ M ABA alone or combined with 50 or 100 μM hydroxycinnamic acids. Statistical differences were assessed using one-way ANOVA. Post-hoc comparisons were conducted using Tukey's test, and different groups were indicated by different letters (adjusted P value < 0.05).

activity observed by the addition of 0.25 μM ABA in presence of CsPYL1 is recovered at 25 μM ligand concentration (Fig. 3D).

We were able to determine the crystal structure of SlPYL1 bound to FER-PHE (Fig. 3E and Fig. S1). This structure aligns well with the initial design strategy to disrupt gate closure, as the aromatic amino acid stabilizes the gate in an open position. However, unlike ANT, where the molecule extends to occupy the position of HAB1 Trp 385 in the Trp-lock, the conjugate molecule extends in the opposite direction to make contacts with Leu 196 and the hydrophobic moieties of the side chain of His 152 and Arg 153 (Fig. 3E).

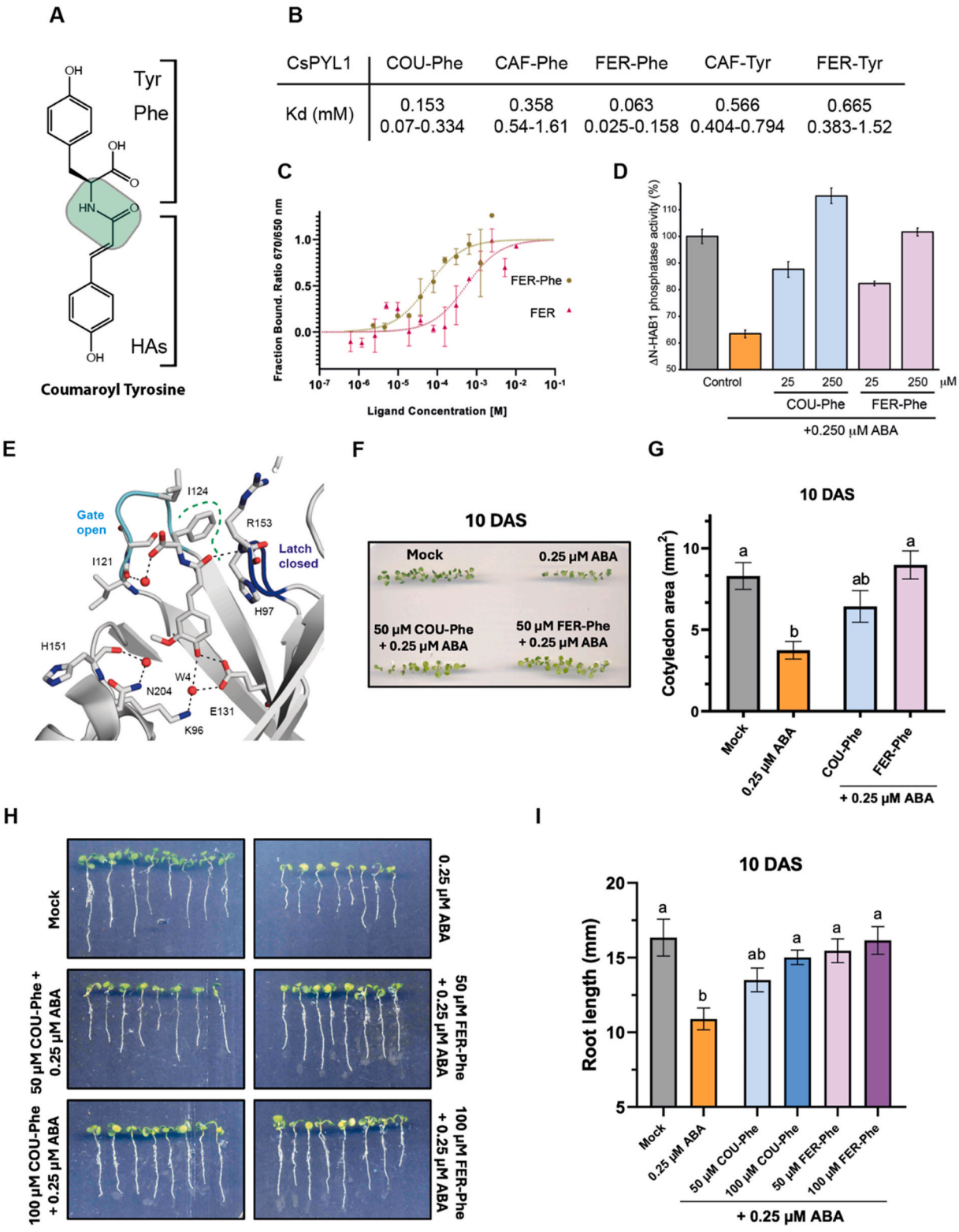
3.4. In vivo activity of COU-Phe and FER-Phe in germination and seedling establishment inhibition assays

We evaluated the in vivo activity of COU-Phe and FER-Phe in wild-type (WT) Arabidopsis plants using seedling establishment inhibition assays (Fig. 3F and G). To assess their effects, we compared ABA-mediated inhibition of cotyledon growth with the inhibition observed in the presence of 0.25 μM ABA and either COU-Phe or FER-Phe. Our results showed a significant recovery of cotyledon growth in the presence of 50 μM FER-Phe. This effect was also evident in root length measurements taken 10 days after sowing, following the seedling establishment in a medium supplemented with ABA and each of the conjugated molecules (Fig. 3H and I).

4. Discussion

ABA antagonists are valuable biotechnological tools for modulating the ABA signaling pathway. By inhibiting ABA's natural effects, antagonists provide a means to finely control processes such as stomatal closure, seed dormancy, and stress responses (Takeuchi et al., 2014, 2018; Vaidya et al., 2021; Wang et al., 2024). Control of ABA homeostasis is also critical for stress tolerance in the reproductive stage of cereals (Ji et al., 2011). Recently, disruption of ABA signaling through antabactin treatment proved useful in overcoming the high-temperature inhibition of seed germination (Eckhardt et al., 2024). Thus, antagonists open opportunities for agricultural applications, such as enhancing photosynthesis and, consequently, plant performance, when water is not limited or improving seed germination rates under unfavorable conditions such as thermoinhibition, by counteracting excessive ABA activity. Natural ABA antagonists offer significant advantages over synthetic alternatives. Since they are derived from naturally occurring molecules, they are more likely to be environmentally friendly, biodegradable, and less toxic to plants, animals, and humans. Moreover, their use reduces the reliance on complex and often expensive chemical synthesis processes, making them more accessible and sustainable for large-scale agricultural applications.

Using structure-based drug-oriented technologies, we have discovered that coumaric, ferulic, and caffeic acids bind to the dimeric CsPYL1 ABA binding site, preventing ABA-mediated HAB1 inhibition (Fig. 1). Interestingly, through solving the crystal structures of their complexes with SlPYL1, we demonstrated that their function is based on blocking the structural transition required for the gate loop to close, which is essential for phosphatase binding and triggering ABA mediated response (Melcher et al., 2009). The joined structural analysis and biochemical data reveal how replacing water molecules within the ABA binding pocket creates new hydrogen bond interactions with the ligand and/or hydrophobic interactions, thereby influencing affinities (Figs. 1 and 2). Among the tested molecules, FER exhibits the highest affinity for CsPYL1 which is related to the increased number of hydrophobic interactions in the ABA binding pocket. In addition, we have shown that the action occurs as well in vivo. The data in vivo correlate well with



(caption on next page)

Fig. 3. The scaffold merging approach for the generation of ABA antagonist molecules. (A) The structure of coumaroyl-tyrosine guided the design of the conjugated molecules synthesized by merging hydroxycinnamic acids with the aromatic amino acids Phe and Tyr. The green box highlights the amide scaffold. (B) The K_d (mM) values of the conjugated molecules of the hydroxycinnamic acids for CsPYL1 as determined using fluorescence spectral shift using the Monolith X (NanoTemper Technologies) and performed in duplicates. The confidence values are also indicated. (C) FER-Phe shows a K_d ~10-fold lower than FER for CsPYL1. The X-axes show increasing concentrations of ligand, and the Y-axes show the ratio between the emitted fluorescence at 670 nm and 650 nm. Spectral shift dose-response values were performed in duplicates. The fitting curves were calculated using the Monolith X analysis software. (D) AtHAB1 phosphatase activity in the presence of CsPYL1 and 0.25 μ M ABA and different concentrations of COU-Phe and FER-Phe were incubated at different ABA concentrations. The data are presented as the mean ± standard deviation of four replicates. (E) A view of the ABA-binding pocket in the structures of SlPYL1 in complex with feruloylphenylalanine (FER-Phe). Water-mediated hydrogen bonds ligands and receptors are represented as dashed lines. (F) The conjugated molecules (50 μ M COU-Phe and FER-Phe) restore cotyledon growth in seedlings germinated in the presence of 0.25μ M ABA. Cotyledon growth was scored at 10 d after sowing (das). (G) Quantification of cotyledon area in the experiment described in (F). Bars indicate the mean of 20 cotyledon areas from two independent experiments (N = 10 cotyledon per well from each experiment quantified) ± SE. The cotyledon area was measured in the absence (mock) or presence of 0.25 μ M ABA alone or combined with 50μ M COU-Phe and FER-Phe. Statistical differences were assessed using one-way ANOVA. Post-hoc comparisons were conducted using Tukey's test, and different groups were indicated by different letters (adjusted P value < 0.05). (H) The conjugated molecules (50 and 100 μ M COU-Phe and FER-Phe) restore root length in seedlings germinated in the presence of 0.25μ M ABA. Cotyledon growth was scored at 10 d after sowing (das). (I) Quantification of root length in the experiment described in (H). Bars indicate the mean of 16 values from two independent experiments (N = 8 roots per well in each experiment quantified) ± SE. The root length was measured in the absence (mock) or presence of 0.25 μ M ABA alone or combined with 50 or 100μ M COU-Phe and FER-Phe. Statistical differences were assessed using one-way ANOVA. Post-hoc comparisons were conducted using Tukey's test, and different groups were indicated by different letters (adjusted P value < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

biochemical data showing that FER is more potent ABA modulator than CAF or COU. Collectively, our results suggest that the observed action in vivo occurs through the interaction of hydroxycinnamic acids with the PYR/PYL receptors.

Many byproducts of the food industry are rich in polyphenols due to their plant-based origin. Among them, spent coffee grounds, a primary byproduct of the brewing process, contain phenolic acids that are easily extracted with water (Dorsey and Jones, 2017). In particular, Monente et al. (2015) analyzed the total phenolic content in spent coffee ground extracts and identified free and bound forms of caffeic, ferulic, and p-coumaric acids. The large volume of coffee produced globally generates significant agro-industrial by-products. These waste materials represent an underutilized and sustainable source of natural compounds with potential ABA-antagonistic activity. By repurposing such by-products in agriculture, it may be possible to modulate ABA responses in crops in an environmentally friendly and cost-effective manner. Thus, offering a sustainable application of these by-products that aligns with circular economy principles and could contribute to more sustainable crop management practices. Our data suggest that the antagonist activity of hydroxycinnamic acids could explain the empirically observed benefits of coffee grounds in promoting ornamental plant health, potentially by alleviating ABA-mediated growth inhibition. This study has identified naturally occurring phytochemicals capable of suppressing ABA signaling and, given the ubiquity of hydroxycinnamic derivatives in plants, lends mechanistic support to this hypothesis.

In contrast to the rich source of natural compounds that can antagonize ABA, we are not aware of natural compounds (outside of ABA) that might act as agonists of ABA receptors. At least in one different hormonal pathway, such a situation occurs. Thus, in the case of jasmonic acid (JA) signaling, the COI1 receptor is activated both by the endogenous ligand JA-Ile and by the bacterial phytotoxin coronatine, produced by biotrophic bacteria (Sheard et al., 2010). To date, no molecules playing an analogous role to coronatine in JA signaling have been identified in ABA signaling. However, certain pathogenic fungi produce molecules structurally related to ABA, such as pyrenophoric acid, which can be converted into ABA through the plant's ABA biosynthetic pathway, thereby inhibiting seed germination (Lozano-Juste et al., 2019).

The affinities observed for coumaric, ferulic, and caffeic acids are lower than those reported for other synthetic antagonists such as ANT (Vaidya et al., 2021). Indeed, ANT shows four orders of magnitude higher affinity than those observed for ferulic acid, which is the best of the hydroxycinnamic acids tested. However, hydroxycinnamic acids are found in various plants at high concentrations, particularly in grapevine or coffee (Sikuten et al., 2020). Their concentrations in plant tissues vary depending on the species and environmental conditions. For instance, hydroxycinnamic acids comprise 5–10 % by weight of roasted coffee beans (Dorsey and Jones, 2017). Thus, the natural concentrations of these compounds are comparable to the affinities observed in our experiments, suggesting they could act as modulators of ABA signaling in vivo. ABA-mediated complete stomatal closure is crucial for preventing dehydration during stress conditions, but ABA also restricts photosynthesis by limiting CO_2 uptake (Yang et al., 2019) and CO_2 mesophyll conductance (Pizzio et al., 2024). The presence of natural ABA antagonists in plants, such as niacin (Infantes et al., 2022) or hydroxycinnamic acids, might provide a regulatory mechanism to balance this trade-off. By modulating ABA signaling, these antagonists would help fine-tune stomatal closure, preventing excessive water loss while maintaining sufficient photosynthetic activity to support plant growth and survival. Thus, natural ABA antagonists might provide a natural mechanism to balance stress responses, water conservation and photosynthesis.

To further enhance the activity of hydroxycinnamic acids, we designed a series of antagonists by modifying their carboxylate moieties. These structural modifications were intended to disrupt the Trp lock (Figs. 1A and 3), impede gate closure of the PYR/PYL receptor, and inhibit receptor-PP2C interactions, mirroring the effects of known antagonist compounds such as PanMe and ANT (Takeuchi et al., 2018; Vaidya et al., 2021). Crystallographic analysis confirmed their interactions at the ABA binding site. Both in vitro and in vivo assays demonstrated superior efficacy compared to COU, CAF, and FER, supporting their potential as optimized ABA antagonists. Further experiments in Arabidopsis or crop species are needed to evaluate their ability to promote germination under supraoptimal temperatures, for example (Eckhardt et al., 2024). Given that thermoinhibition disrupts synchronous germination during heat stress, chemical disruption of ABA signaling represents a promising strategy to mitigate this effect (Eckhardt et al., 2024). Additionally, in cereals under drought stress, targeted application of natural or synthetic antagonists during specific reproductive stages could improve spike fertility and prevent pollen abortion (Ji et al., 2011). Thus, the use of natural metabolites modulating ABA signaling (or synthetic derivatives) represents another tool to face climate change.

CRediT authorship contribution statement

Javier Merino: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **María Rivera-Moreno:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Mar Bono:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Diego Núñez-Villanueva:** Writing – review & editing, Visualization, Methodology, Investigation. **Ana González-Vega:** Investigation. **Cristian Mayordomo:** Investigation. **Lourdes Infantes:** Writing –

review & editing, Methodology, Investigation, Conceptualization. **Rupesh Chikhale:** Writing – review & editing, Supervision, Methodology. **Pedro L. Rodríguez:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Armando Albert:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Funding

Agencia Estatal de Investigación of the Spanish Ministry of Science and Innovation with grant number PID2020-119805RB-I00 and PID2023-153108OB-I00 funded by MCIN/AEI/10.13039/501100011033 and the ERDF ("A way of making Europe") (A. A.)

Agencia Estatal de Investigación of the Spanish Ministry of Science and Innovation with grant number PID2020-113100RB-I00 and PID2023-147322OB-I00 funded by MCIN/AEI/10.13039/501100011033 and the ERDF ("A way of making Europe") (P. L. R.)

Agencia Estatal de Investigación of the Spanish Ministry of Science and Innovation with grant number TED2021-129867B-C21 (P.L.R.) and TED2021-132202B-I00 (A.A.) funded by MCIU/AEI/10.13039/501100011033 and by the European Union NextGenerationEU/PRTR.

Fundación General CSIC ComFuturo fellowship - European Union's Horizon 2020 research and innovation program, under the Marie Skłodowska-Curie grant agreement No. 101034263. (D.N.-V).

Agencia Estatal de Investigación of the Spanish Ministry of Science and Innovation with Ramón y Cajal fellowship RYC2022-035730-I funded by MCIN/AEI/10.13039/501100011033 and FSE+ (D.N.-V).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Data collection experiments were performed at XALOC beamline at ALBA Synchrotron with the collaboration of ALBA staff.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.110155.

Data availability

The atomic coordinates and structure factors of the complexes between SlPYL1 and COU, CAF, FER and FER-Phe have been deposited in the Protein Data Bank (https://www.rcsb.org/) with PDB IDs 9QK3, 9QK5, 9QK4 and 9QK6 respectively.

References

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W., et al., 2010. PHENIX: a comprehensive Python-based system for macromolecular structure solution title. Acta Crystallogr. Sect. D Biol. Crystallogr. 66, 213–221.

Agirre, J., Atanasova, M., Bagdonas, H., Ballard, C.B., Basle, A., Beilsten-Edmands, J., Borges, R.J., Brown, D.G., Burgos-Marmol, J.J., Berrisford, J.M., et al., 2023. The CCP4 suite: integrative software for macromolecular crystallography. Acta Crystallogr. D Struct. Biol. 79, 449–461.

Barros, E.P., Ries, B., Champion, C., Rieder, S.R., Riniker, S., 2023. Accounting for solvation correlation effects on the thermodynamics of water networks in protein cavities. J. Chem. Inf. Model. 63, 1794–1805.

Bono, M., Ferrer-Gallego, R., Pou, A., Rivera-Moreno, M., Benavente, J.L., Mayordomo, C., Deis, L., Carbonell-Bejerano, P., Pizzio, G.A., Navarro-Paya, D., et al., 2024. Chemical activation of ABA signaling in grapevine through the iSB09 and AMF4 ABA receptor agonists enhances water use efficiency. Physiol. Plantarum 176, e14635.

Canto-Pastor, A., Kajala, K., Shaar-Moshe, L., Manzano, C., Timilsena, P., De Bellis, D., Gray, S., Holbein, J., Yang, H., Mohammad, S., et al., 2024. A suberized exodermis is required for tomato drought tolerance. Nat. Plants 10, 118–130.

Cao, M., Liu, X., Zhang, Y., Xue, X., Zhou, X.E., Melcher, K., Gao, P., Wang, F., Zeng, L., Zhao, Y., et al., 2013. An ABA-mimicking ligand that reduces water loss and promotes drought resistance in plants. Cell Res. 23, 1043–1054.

Daniel-Mozo, M., Albert, A., 2021. It takes two to tango: unraveling a new post-translational modification involved in SnRK2.6 activation. Mol. Plant 14, 1779–1781.

DeLano, 2002. In: Schrödinger, L. (Ed.), The Pymol Molecular Graphics System.

Dorsey, B.M., Jones, M.A., 2017. In: Galanakis, C.M. (Ed.), Handbook of Coffee Processing By-Products. Academic Press, pp. 27–62.

Dupeux, F., Santiago, J., Betz, K., Twycross, J., Park, S.Y., Rodriguez, L., Gonzalez-Guzman, M., Jensen, M.R., Krasnogor, N., Blackledge, M., et al., 2011. A thermodynamic switch modulates abscisic acid receptor sensitivity. EMBO J. 30, 4171–4184.

Eckhardt, J., Vaidya, A., Cutler, S., 2024. Chemical disruption of ABA signaling overcomes high-temperature inhibition of seed germination and enhances seed priming responses. PLoS One 19, e0315290.

Emsley, P., Cowtan, K., 2004. Coot: model-building tools for molecular graphics title. Acta Crystallogr. Sect. D Biol. Crystallogr. 60, 2126–2132.

Erb, M., Kliebenstein, D.J., 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol. 184, 39–52.

Fujii, H., Chinnusamy, V., Rodrigues, A., Rubio, S., Antoni, R., Park, S.Y., Cutler, S.R., Sheen, J., Rodriguez, P.L., Zhu, J.K., 2009. In vitro reconstitution of an abscisic acid signalling pathway. Nature 462, 660–664.

Furihata, T., Maruyama, K., Fujita, Y., Umezawa, T., Yoshida, R., Shinozaki, K., Yamaguchi-Shinozaki, K., 2006. Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. Proc. Natl. Acad. Sci. USA 103, 1988–1993.

Hao, Q., Yin, P., Li, W., Wang, L., Yan, C., Lin, Z., Wu, J.Z., Wang, J., Yan, S.F., Yan, N., 2011. The molecular basis of ABA-independent inhibition of PP2Cs by a subclass of PYL proteins. Mol. Cell 42, 662–672.

Hewage, K.A.H., Yang, J.F., Wang, D., Hao, G.F., Yang, G.F., Zhu, J.K., 2020. Chemical manipulation of abscisic acid signaling: a new approach to abiotic and biotic stress management in agriculture. Adv. Sci. (Weinh.) 7, 2001265.

Infantes, L., Rivera-Moreno, M., Daniel-Mozo, M., Benavente, J.L., Ocana-Cuesta, J., Coego, A., Lozano-Juste, J., Rodriguez, P.L., Albert, A., 2022. Structure-based modulation of the ligand sensitivity of a tomato dimeric abscisic acid receptor through a glu to asp mutation in the latch loop. Front. Plant Sci. 13, 884029.

Ji, X., Dong, B., Shiran, B., Talbot, M.J., Edlington, J.E., Hughes, T., White, R.G., Gubler, F., Dolferus, R., 2011. Control of abscisic acid catabolism and abscisic acid homeostasis is important for reproductive stage stress tolerance in cereals. Plant Physiol. 156, 647–662.

Jones, G., Willett, P., Glen, R.C., Leach, A.R., Taylor, R., 1997. Development and validation of a genetic algorithm for flexible docking. J. Mol. Biol. 267, 727–748.

Korb, O., Stutzle, T., Exner, T.E., 2009. Empirical scoring functions for advanced protein-ligand docking with PLANTS. J. Chem. Inf. Model. 49, 84–96.

Lin, Z., Li, Y., Wang, Y., Liu, X., Ma, L., Zhang, Z., Mu, C., Zhang, Y., Peng, L., Xie, S., et al., 2021. Initiation and amplification of SnRK2 activation in abscisic acid signaling. Nat. Commun. 12, 2456.

Lin, Z., Li, Y., Zhang, Z., Liu, X., Hsu, C.C., Du, Y., Sang, T., Zhu, C., Wang, Y., Satheesh, V., et al., 2020. A RAF-SnRK2 kinase cascade mediates early osmotic stress signaling in higher plants. Nat. Commun. 11, 613.

Lozano-Juste, J., Garcia-Maquilon, I., Brea, J., Pina, R., Albert, A., Rodriguez, P.L., Loza, M.I., 2021. Identification of ABA receptor agonists using a multiplexed high-throughput chemical screening. Methods Mol. Biol. 2213, 99–111.

Lozano-Juste, J., Garcia-Maquilon, I., Ruiz-Partida, R., Rodriguez, P.L., 2020. Drug discovery for thirsty crops. Trends Plant Sci. 25, 844–846.

Lozano-Juste, J., Infantes, L., Garcia-Maquilon, I., Ruiz-Partida, R., Merilo, E., Benavente, J.L., Velazquez-Campoy, A., Coego, A., Bono, M., Forment, J., et al., 2023. Structure-guided engineering of a receptor-agonist pair for inducible activation of the ABA adaptive response to drought. Sci. Adv. 9, eade9948.

Lozano-Juste, J., Masi, M., Cimmino, A., Clement, S., Fernandez, M.A., Antoni, R., Meyer, S., Rodriguez, P.L., Evidente, A., 2019. The fungal sesquiterpenoid pyrenophoric acid B uses the plant ABA biosynthetic pathway to inhibit seed germination. J. Exp. Bot. 70, 5487–5494.

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A., Grill, E., 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science 324, 1064–1068.

Martinez-Ripoll, M., Albert, A., 2023. Continuous development in macromolecular crystallography with CCP4. Acta Crystallogr. D Struct. Biol. 79, 447–448.

Melcher, K., Ng, L.M., Zhou, X.E., Soon, F.F., Xu, Y., Suino-Powell, K.M., Park, S.Y., Weiner, J.J., Fujii, H., Chinnusamy, V., et al., 2009. A gate-latch-lock mechanism for hormone signalling by abscisic acid receptors. Nature 462, 602–608.

Melcher, K., Xu, Y., Ng, L.M., Zhou, X.E., Soon, F.F., Chinnusamy, V., Suino-Powell, K.M., Kovach, A., Tham, F.S., Cutler, S.R., et al., 2010a. Identification and mechanism of ABA receptor antagonism. Nat. Struct. Mol. Biol. 17, 1102–1108.

Melcher, K., Zhou, X.E., Xu, H.E., 2010b. Thirsty plants and beyond: structural mechanisms of abscisic acid perception and signaling. Curr. Opin. Struct. Biol. 20, 722–729.

Miao, C., Xiao, L., Hua, K., Zou, C., Zhao, Y., Bressan, R.A., Zhu, J.K., 2018. Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. Proc. Natl. Acad. Sci. USA 115, 6058–6063.

Miyazono, K., Miyakawa, T., Sawano, Y., Kubota, K., Kang, H.J., Asano, A., Miyauchi, Y., Takahashi, M., Zhi, Y., Fujita, Y., et al., 2009. Structural basis of abscisic acid signalling. Nature 462, 609–614.

Monente, C., Ludwig, I.A., Irigoyen, A., De Pena, M.P., Cid, C., 2015. Assessment of total (free and bound) phenolic compounds in spent coffee extracts. J. Agric. Food Chem. 63, 4327–4334.

Moreno-Alvero, M., Yunta, C., Gonzalez-Guzman, M., Lozano-Juste, J., Benavente, J.L., Arbona, V., Menendez, M., Martinez-Ripoll, M., Infantes, L., Gomez-Cadenas, A., et al., 2017. Structure of ligand-bound intermediates of crop ABA receptors highlights PP2C as necessary ABA Co-receptor. Mol. Plant 10, 1250–1253.

Nishimura, N., Hitomi, K., Arvai, A.S., Rambo, R.P., Hitomi, C., Cutler, S.R., Schroeder, J. I., Getzoff, E.D., 2009. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1. Science 326, 1373–1379.

Okamoto, M., Peterson, F.C., Defries, A., Park, S.Y., Endo, A., Nambara, E., Volkman, B. F., Cutler, S.R., 2013. Activation of dimeric ABA receptors elicits guard cell closure, ABA-regulated gene expression, and drought tolerance. Proc. Natl. Acad. Sci. USA 110, 12132–12137.

Park, S.Y., Fung, P., Nishimura, N., Jensen, D.R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T.F., et al., 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science 324, 1068–1071.

Peirats-Llobet, M., Han, S.K., Gonzalez-Guzman, M., Jeong, C.W., Rodriguez, L., Belda-Palazon, B., Wagner, D., Rodriguez, P.L., 2016. A direct link between abscisic acid sensing and the chromatin-remodeling ATPase BRAHMA via core ABA signaling pathway components. Mol. Plant 9, 136–147.

Pizzio, G.A., Mayordomo, C., Illescas-Miranda, J., Coego, A., Bono, M., Sanchez-Olvera, M., Martin-Vasquez, C., Samantara, K., Merilo, E., Forment, J., et al., 2024. Basal ABA signaling balances transpiration and photosynthesis. Physiol. Plantarum 176, e14494.

Rodriguez, P.L., Lozano-Juste, J., Albert, A., 2019. PYR/PYL/RCAR ABA receptors. Adv. Bot. Res. 92, 51–82.

Rodriguez-Larrea, D., Perez-Jimenez, R., Sanchez-Romero, I., Delgado-Delgado, A., Fernandez, J.M., Sanchez-Ruiz, J.M., 2010. Role of conservative mutations in protein multi-property adaptation. Biochem. J. 429, 243–249.

Rothwell, J.A., Perez-Jimenez, J., Neveu, V., Medina-Remon, A., M'Hiri, N., Garcia-Lobato, P., Manach, C., Knox, C., Eisner, R., Wishart, D.S., et al., 2013. Phenol-explorer 3.0: a major update of the phenol-explorer database to incorporate data on the effects of food processing on polyphenol content. Database (Oxford) 2013, bat070.

Santiago, J., Dupeux, F., Round, A., Antoni, R., Park, S.Y., Jamin, M., Cutler, S.R., Rodriguez, P.L., Marquez, J.A., 2009a. The abscisic acid receptor PYR1 in complex with abscisic acid. Nature 462, 665–668.

Santiago, J., Rodrigues, A., Saez, A., Rubio, S., Antoni, R., Dupeux, F., Park, S.Y., Marquez, J.A., Cutler, S.R., Rodriguez, P.L., 2009b. Modulation of drought resistance by the abscisic acid receptor PYL5 through inhibition of clade A PP2Cs. Plant J. : Cell Mol. Biol. 60, 575–588.

Serra, O., Geldner, N., 2022. The making of suberin. New Phytol. 235, 848–866.

Sheard, L.B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T.R., Kobayashi, Y., Hsu, F.F., Sharon, M., Browse, J., et al., 2010. Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468, 400–405.

Sikuten, I., Stambuk, P., Andabaka, Z., Tomaz, I., Markovic, Z., Stupic, D., Maletic, E., Kontic, J.K., Preiner, D., 2020. Grapevine as a rich source of polyphenolic compounds. Molecules 25.

Soma, F., Takahashi, F., Kidokoro, S., Kameoka, H., Suzuki, T., Uga, Y., Shinozaki, K., Yamaguchi-Shinozaki, K., 2023. Constitutively active B2 Raf-like kinases are required for drought-responsive gene expression upstream of ABA-activated SnRK2 kinases. Proc. Natl. Acad. Sci. USA 120, e2221863120.

Soon, F.F., Ng, L.M., Zhou, X.E., West, G.M., Kovach, A., Tan, M.H., Suino-Powell, K.M., He, Y., Xu, Y., Chalmers, M.J., et al., 2012. Molecular mimicry regulates ABA signaling by SnRK2 kinases and PP2C phosphatases. Science 335, 85–88.

Takahashi, Y., Zhang, J., Hsu, P.K., Ceciliato, P.H.O., Zhang, L., Dubeaux, G., Munemasa, S., Ge, C., Zhao, Y., Hauser, F., et al., 2020. MAP3Kinase-dependent SnRK2-kinase activation is required for abscisic acid signal transduction and rapid osmotic stress response. Nat. Commun. 11, 12.

Takeuchi, J., Mimura, N., Okamoto, M., Yajima, S., Sue, M., Akiyama, T., Monda, K., Iba, K., Ohnishi, T., Todoroki, Y., 2018. Structure-based chemical design of abscisic acid antagonists that block PYL-PP2C receptor interactions. ACS Chem. Biol. 13, 1313–1321.

Takeuchi, J., Okamoto, M., Akiyama, T., Muto, T., Yajima, S., Sue, M., Seo, M., Kanno, Y., Kamo, T., Endo, A., et al., 2014. Designed abscisic acid analogs as antagonists of PYL-PP2C receptor interactions. Nat. Chem. Biol. 10, 477–482.

Vaidya, A.S., Helander, J.D.M., Peterson, F.C., Elzinga, D., Dejonghe, W., Kaundal, A., Park, S.Y., Xing, Z., Mega, R., Takeuchi, J., et al., 2019. Dynamic control of plant water use using designed ABA receptor agonists. Science 366.

Vaidya, A.S., Peterson, F.C., Eckhardt, J., Xing, Z., Park, S.Y., Dejonghe, W., Takeuchi, J., Pri-Tal, O., Faria, J., Elzinga, D., et al., 2021. Click-to-lead design of a picomolar ABA receptor antagonist with potent activity in vivo. Proc. Natl. Acad. Sci. USA 118.

Vonrhein, C., Tickle, I.J., Flensburg, C., Keller, P., Paciorek, W., Sharff, A., Bricogne, G., 2018. Advances in automated data analysis and processing within autoPROC, combined with improved characterisation, mitigation and visualisation of the anisotropy of diffraction limits using STARANISO. Acta Crystallogr. A 74.

Wang, Z.Z., Cao, M.J., Yan, J., Dong, J., Chen, M.X., Yang, J.F., Li, J.H., Ying, R.N., Gao, Y.Y., Li, L., et al., 2024. Stabilization of dimeric PYR/PYL/RCAR family members relieves abscisic acid-induced inhibition of seed germination. Nat. Commun. 15, 8077.

Weng, J.K., Ye, M., Li, B., Noel, J.P., 2016. Co-evolution of hormone metabolism and signaling networks expands plant adaptive plasticity. Cell 166, 881–893.

Yang, Z., Liu, J., Poree, F., Schaeufele, R., Helmke, H., Frackenpohl, J., Lehr, S., von Koskull-Doring, P., Christmann, A., Schnyder, H., et al., 2019. Abscisic acid receptors and coreceptors modulate plant water use efficiency and water productivity. Plant Physiol. 180, 1066–1080.

Zagoskina, N.V., Zubova, M.Y., Nechaeva, T.L., Kazantseva, V.V., Goncharuk, E.A., Katanskaya, V.M., Baranova, E.N., Aksenova, M.A., 2023. Polyphenols in plants: structure, biosynthesis, abiotic stress regulation, and practical applications. Int. J. Mol. Sci. 24 (Review).

Zhang, X., Mou, Z., 2009. Extracellular pyridine nucleotides induce PR gene expression and disease resistance in arabidopsis. Plant J. : Cell Mol. Biol. 57, 302–312.