RESEARCH PAPER

PYL8 ABA receptors of *Phoenix dactylifera* play a crucial role in response to abiotic stress and are stabilized by ABA

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Abstract

The identification of those prevalent abscisic acid (ABA) receptors and molecular mechanisms that trigger drought adaptation in crops well adapted to harsh conditions such as date palm (*Phoenix dactylifera*, Pd) sheds light on plant–environment interactions. We reveal that PdPYL8-like receptors are predominantly expressed under abiotic stress, with Pd27 being the most expressed receptor in date palm. Therefore, subfamily I PdPYL8-like receptors have been selected for ABA signaling during abiotic stress response in this crop. Biochemical characterization of PdPYL8-like and PdPYL1-like receptors revealed receptor- and ABA-dependent inhibition of PP2Cs, which triggers activation of the *pRD29B-LUC* reporter in response to ABA. PdPYLs efficiently abolish PP2C-mediated repression of ABA signaling, but loss of the Trp lock in the seed-specific AHG1-like phosphatase PdPP2C79 markedly impairs its inhibition by ABA receptors. Characterization of Arabidopsis transgenic plants that express PdPYLs shows enhanced ABA signaling in seed, root, and guard cells. Specifically, Pd27-overexpressing plants showed lower ABA content and were more efficient than the wild type in lowering transpiration at negative soil water potential, leading to enhanced drought tolerance. Finally, PdPYL8-like receptors accumulate after ABA treatment, which suggests that ABA-induced stabilization of these receptors operates in date palm for efficient boosting of ABA signaling in response to abiotic stress.

Keywords: ABA receptor, abiotic stress, abscisic acid, AHG1, date palm, *Phoenix dactylifera*, PP2C, PYL8.

Introduction

Among monocotyledons, the palm family plants (Arecaceae or Palmae) are second in economic importance after the grass family (Poaceae) and, among the palm crops, the top three palm species are African oil palm (*Elaeis guineensis*), date palm (*Phoenix dactylifera*), and coconut (*Cocos nucifera*). Date palm (*P. dactylifera* L., 2*n*=36) is thought to be native to the Arabian Peninsula from where it was introduced to northern India, North Africa, and southern Spain (Al-Mssallem *et al.*, 2013). Date palm used to be the main nutrient for the population of the Arabian Peninsula and currently is a major food crop in the Middle East, northern Africa, and other places with suitable climate (Yin *et al.*, 2012). Additionally, it is a sociocultural symbol in many countries, with a long agriculture history, being one of oldest domesticated trees (Al-Mssallem *et al.*, 2013).

Date palm is a plant species adapted for growth under dry conditions, and recent studies indicate that heat and drought stresses only slightly affect photosynthesis (Arab *et al.*, 2016). Fast guard cell abscisic acid (ABA) signaling is present in date palm to limit transpirational water loss, and the *P. dactylifera* (Pd) SLAC1-type anion channel is regulated by the ABA kinase PdOST1 (Müller *et al.*, 2017). In the presence of nitrate at the extracellular face of the anion channel, ABA enhanced and accelerated stomatal closure (Müller *et al.*, 2017). Additionally, the date palm genome displays an expanded late embryogenesis abundant (LEA) gene family, whose ABA-induced expression is important for resistance to abiotic stress, particularly LEA2 genes that are ubiquitously expressed (Al-Mssallem *et al.*, 2013). Given that ABA plays a key role for plant adaptation to drought and that date palm is well adapted to survive and produce fruits under harsh environmental conditions, the characterization of core ABA signaling components in Pd is of great interest. ABA signaling is initiated by ABA perception through PYRABACTIN RESISTANCE1 (PYR1)/ PYR1-LIKE (PYL)/REGULATORY COMPONENTS OF ABA RECEPTORS (RCAR) receptors (Park *et al.*, 2009; Ma *et al.*, 2009; Santiago *et al.*, 2009; Rodriguez *et al.*, 2019), which leads to interaction with and inactivation of clade A protein phosphatase type 2Cs (PP2Cs), such as ABA INSENSITIVE 1 (ABI1) and ABI2, HYPERSENSITIVE TO ABA (HAB1) and HAB2, and PROTEIN PHOSPHATASE 2CA/ABA-HYPERSENSITIVE GERMINATION 3 (PP2CA/AHG3), thereby relieving their inhibition on three ABA-activated SNF1-related protein kinases (SnRK2s) termed subclass III SnRK2s, namely SnRK2.2/SnRK2D, 2.3/I, and 2.6/E/OST1 (Umezawa *et al.*, 2009; Vlad *et al.*, 2009).

The discovery of the 14 member gene family of PYR/ PYL/RCAR ABA receptors in *Arabidopsis thaliana* (Ma *et al.*, 2009; Park *et al.*, 2009; Santiago *et al.*, 2009) has enabled the identification of the multigene families of ABA receptors in different crops. Functional characterization of crop ABA receptors is required to determine the prevailing receptors for abiotic stress adaptation; however, this knowledge is limited (Gonzalez-Guzman *et al.*, 2014; Miao *et al.*, 2018). Crop PYR/

PYL/RCARs belong to multigene families, which are composed of 15 members in tomato (Gonzalez-Guzman *et al.*, 2014), 12 in rice (He *et al.*, 2014), 13 in maize (He *et al.*, 2018), nine in wheat (Mega *et al.*, 2019), nine in barley (Seiler *et al.*, 2014), and 11 in sweet orange (Arbona *et al.*, 2017). The receptors encoded in these genomes are distributed in three distinct subfamilies based on amino acid sequence identity, which are reminiscent of the Arabidopsis PYR1/PYL1-like (clade III), PYL4-like (clade II), and PYL8-like subfamilies (clade I) (Rodriguez *et al.*, 2019). However, not all families play similar roles for ABA signaling in different tissues, and specific functions for some members of these subfamilies have emerged in the last years (Antoni *et al.*, 2013; Zhao *et al.*, 2016; Dittrich *et al.*, 2019). In particular, PYL8 plays a non-redundant role for ABA signaling in root, and ABA diminishes ubiquitination of this unique receptor, which leads to its stabilization and accumulation in the presence of ABA (Belda-Palazon *et al.*, 2018). In contrast to Arabidopsis, functional knowledge in crops is scarce and prevents further biotechnological use, for example to regulate transpiration or root architecture in order to better adapt plants to drought.

The genome of date palm (estimated genome length 670 Mb) has been sequenced using different genome assemblies (Al-Dous *et al.*, 2011; Al-Mssallem *et al.*, 2013; Hazzouri *et al.*, 2015, 2019), and different transcriptomic studies have been published (reviewed by Gros-Balthazard *et al.*, 2018). This race for genomic data has enabled the identification and characterization of the sex determination locus (Torres *et al.*, 2018), pathways responsible for fruit maturation (Al-Mssallem *et al.*, 2013; Hazzouri *et al.*, 2019), and elucidation of the domestication history and diversity (Hazzouri *et al.*, 2015; Gros-Balthazard *et al.*, 2018). RNA-seq expression data are available for the different fruit stages, response to different stress treatments, and comparative transcriptome analysis of oil palm and date palm mesocarp (Bourgis *et al.*, 2011; Yin *et al.*, 2012; Yaish *et al.*, 2017; Al-Harrasi *et al.*, 2018; Hazzouri *et al.*, 2019; Xiao *et al.*, 2019). Genomic resources have been used during this work to identify the Pd ABA receptor family and pinpoint the major Pd ABA receptors expressed during ABA treatment, salt, heat, drought, or combined heat–drought conditions (Müller *et al.*, 2017; Safronov *et al.*, 2017). As a result, we reveal that the PYL8-like ABA receptors of *P. dactylifera* play a major role in response to abiotic stress, and their accumulation is promoted by ABA through a post-transcriptional mechanism. Functional characterization of *Phoenix* ABA receptors indicates that inhibition of clade A PP2Cs is crucial for drought tolerance of date palm.

Materials and methods

Plant material and growth conditions

Two-year-old date palm seedlings were cultivated at 22/16 °C, 50% relative humidity, 12 h light regime. For ABA treatment, single cut pinnae

were treated for 2 h with CO_2-free air in darkness to fully open stomata, and then illuminated for 4 h at a photon flux rate of 620 μmol m^{-2} s^{-1}. Following this, 25 μM ABA was fed via the petiole. After 2 h, RNA was extracted from the pinnae. The control was treated equally with ABA-free solution. RNA was extracted from leaves, and RNA-seq library generation and sequencing were as described by Müller *et al.* (2017). For heat, drought, and heat+drought treatments, plants were maintained under greenhouse conditions and transferred to growth chambers for stress treatments. In growth chambers, plants were first acclimatized for 2 weeks (16/8 h photoperiod and 20/15 °C, 70% relative humidity, at a photon flux rate of 200–300 μmol m^{-2} s^{-1} at leaf level). Experiments were carried out in two batches. In the first batch, plants were irrigated every second day (well-watered) and exposed to different growth temperatures: 20/15 °C (70% relative humidity) or 35/15 °C (60–70% relative humidity) for 2 weeks, followed by harvesting 6 h after the onset of light. In the second batch, watering was stopped after the acclimation period (drought conditions), and plants grown at 35 °C were harvested after 4–5 d (drought+heat), whereas plants grown at 20 °C were collected after 7–8 d (drought). For salt treatment, pots were flooded with salt solution for 1 h every second day starting from day 2. After 2 weeks, RNA was extracted from plants treated with salt-free solution, 200 mM salt solution, or 600 mM salt solution. RNA-seq library generation and sequencing was performed as described by Safronov *et al.* (2017). Root and fruit expression data for date palm have been reported by Xiao *et al.*, (2019) and Hazzouri *et al.* (2019), respectively. Briefly, germinated root tips (from 12-week-old seedlings) were sampled for RNA extraction. Total RNA was extracted from 75 mg of plant material. Fruits of the Khalas variety were sampled at 45, 75, 105, 120, and 135 days after pollination (DAP). *Arabidopsis thaliana* plants were grown as described by Planes *et al.* (2015).

Transpiration assay

Transpiration was measured in 16 plants per genotype that were grown in individual Jiffy peat plugs (Jiffy-7 peat pellets, Semillas Batlle S.A., Barcelona, Spain) under different soil water potential conditions according to de Ollas *et al.* (2019). Plants were kept under well-watered conditions for 4 weeks in a growth chamber (Equitec model EGCS 351 3S HR, Getafe, Spain) with a day/night temperature of 23/18 °C, an 8 h light photoperiod (100 μmol m^{-2} s^{-1}), and a relative humidity of 60–65%. To set up the drought conditions, plants were randomly divided into four groups of four plants and differentially watered to cover the soil water content range from well-watered conditions to plant wilting (de Ollas *et al.*, 2019). To characterize transpiration, individual plugs were weighed every 24 h to account for transpiration water loss of plants and individually normalized to leaf area. Transpiration was measured on two consecutive days before harvesting. Plant tissue of each individual plant was immediately frozen in liquid N_2 and lyophilized to analyze endogenous ABA content.

ABA quantification

ABA analysis was performed according to Durgbanshi *et al.* (2005) with slight modifications. Briefly, 0.1 g of dry plant material was extracted in 2 ml of distilled H_2O after spiking with 25 μl of a 2 mg l^{-1} solution of d6-ABA as internal standard. After centrifugation (10 000 *g* at 4 °C), supernatants were recovered, and the pH was adjusted to 3.0 with 30% acetic acid. The acidified water extract was partitioned twice against 3 ml of di-ethyl ether. The organic layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan, Saint Herblain Cedex, France). The dry residue was then re-suspended in a 9:1 H_2O:MeOH solution by sonication. The resulting solution was filtered and directly injected into a UPLC system (Waters Acquity SDS, Waters Corp., Milford, MA, USA) interfaced to a TQD triple quadrupole (Micromass Ltd, Manchester, UK) mass spectrometer through an

orthogonal Z-spray electrospray ion source. Separations were carried out on a Gravity C18 column (50×2.1 mm, 1.8 μm, Macherey-Nagel GmbH, Germany) using a linear gradient of MeOH and H_2O supplemented with 0.1% acetic acid at a flow rate of 300 μl min^{-1}. Transitions for ABA/d6-ABA (263>153/269>159) were monitored in negative ionization mode. Quantitation of plant hormones was achieved by external calibration with known amounts of pure standards using Masslynx v4.1 software.

Generation of Arabidopsis transgenic plants

The indicated pAlligator2-*35S:Pd* and pMDC43-*35S:GFP-Pd* constructs were transferred to *Agrobacterium tumefaciens* C58C1 (pGV2260) (Deblaere *et al.*, 1985) by electroporation and used to transform Columbia wild-type plants by the floral dip method (Clough and Bent, 1998). T_1 transgenic seeds were selected based on seed green fluorescent protein (GFP) fluorescence (pAlligator2) or hygromycin resistance (pMDC43), and sown in soil to obtain the T_2 generation. Homozygous T_3 progeny were used for further studies, and expression of HA-tagged or GFP-tagged protein was verified by immunoblot analysis using anti-HA-hoseradish peroxidase (HRP) or anti-GFP antibodies, respectively.

Constructs

The PdPP2C55, PdPP2C79, Pd15, Pd27, Pd32, Pd44, and Pd957 coding sequences were obtained as synthetic DNA fragments (Invitrogen), which were amplified using the indicated primers (Supplementary Table S1 at *JXB* online), verified by sequencing, and cloned into pCR8/GW/TOPO. In order to obtain recombinant protein, each coding sequence was excised using an *Nco*I–*Eco*RI double digestion and cloned into pETM11.

Chemicals

The ABA agonists used in this work were provided by Dr Sean R. Cutler (University *of* California, Riverside, USA). ABA was obtained from Biosynth (https://www.biosynth.com/).

Transient protein expression in Nicotiana benthamiana

Agrobacterium infiltration of *N. benthamiana* leaves was performed basically as described by Saez *et al.* (2008). For transient expression of GFP–PdPP2C55, PdPP2C79, or Pd ABA receptors, the corresponding pMDC43 binary vector was introduced into *A. tumefaciens* C58C1 (pGV2260) by electroporation and transformed cells were selected in LB plates supplemented with kanamycin (50 μg ml^{-1}). Then, they were grown in liquid LB medium to late exponential phase and cells were harvested by centrifugation and resuspended in 10 mM MES-KOH pH 5.6 containing 10 mM $MgCl_2$ and 150 mM acetosyringone to an $OD_{600\,nm}$ of 1. These cells were mixed with an equal volume of *Agrobacterium* C58C1 (pCH32 35S:p19) expressing the silencing suppressor p19 of *Tomato bushy stunt virus* so that the final density of *Agrobacterium* solution was ~1 (final concentration OD_{600}=0.5 each). Bacteria were incubated for 3 h at room temperature and then injected into young fully expanded leaves of 4-week-old *N. benthamiana* plants. Leaves were examined 48–72 h after infiltration using confocal laser scanning microscopy (CLSM).

Protoplast transfection

We analyzed ABA signaling in Arabidopsis protoplasts basically as described by Fujii *et al.* (2009). Briefly, protoplasts prepared from wild-type Col-0 plants were transfected with the reporter construct pRD29B:LUC, p35S:GUS for normalization, and effector plasmids encoding ABF2, ABF2+HAB1, or ABF2+HAB1+PdPYL proteins. Luciferase (LUC) activity was measured in protein extracts prepared from protoplast

Fig. 1. Cladogram and amino acid sequence alignment of date palm PYR/PYL/RCAR ABA receptors. (A) Cladogram of the multiple sequence alignment of date palm and Arabidopsis receptors, revealing three major subfamilies and the ungrouped Pd91. Sequences of *Citrus sinensis* (CsPYL1, Cs46151)

suspensions 6 h after transfection, in either the absence or presence of 5 μM exogenous ABA that was added 3 h before measuring LUC activity. The activity of the pRD29B:LUC reporter was normalized with p35S:GUS (β-glucuronidase) as described by Fujii *et al.* (2009). To generate the effector plasmids, the coding sequence of ABF2, HAB1, Pd15, Pd27, Pd32, Pd44, or Pd957 was recombined by LR reaction from the pCR8 entry vector to the pXCS destination vector (Witte *et al.*, 2004). The pRD29B:LUC and 35S:GUS pSK constructs were previously described (Christmann *et al.*, 2005; Moes *et al.*, 2008)

Protein extraction, analysis, and immunodetection

Protein extracts for immunodetection experiments were prepared from Arabidopsis transgenic lines expressing HA-tagged or GFP-tagged ABA receptors or GFP-tagged PP2C/ABA receptors transiently expressed in *N. benthamiana* as described by Fernandez *et al.* (2020).

Confocal laser scanning microscopy

Confocal imaging was performed using a Zeiss LSM 780 AxioObserver. Z1 laser scanning microscope with C-Apochromat ×40/1.20 W corrective water immersion objective. The GFP fluorophore was excited and fluorescence emission detected at the indicated wavelengths: 488 nm/500–530 nm, and chlorophyll at 561 nm/685–760 nm. Post-acquisition image processing was performed using ZEN (ZEISS Efficient Navigation) Lite 2012 imaging software and ImageJ (http://imagej.nih.gov).

Seed germination and seedling establishment assays

After surface sterilization of the seeds, stratification was conducted in the dark at 4 °C for 3 d. Approximately 100 seeds of each genotype were sown on Murashige and Skoog (MS) plates supplemented with different ABA concentrations per experiment. To score seed germination, radical emergence was analyzed at 72 h after sowing. Seedling establishment was scored as the percentage of seeds that developed green expanded cotyledons and the first pair of true leaves at 5 d or 7 d. Seedling establishment assays in the presence of ABA agonists were performed in 24-well plates, where ~25 seeds of the indicated genotype (three independent experiments) were sown on wells lacking or supplemented with the indicated concentration of ABA, quinabactin (QB), or AMF4.

Root growth assay

Seedlings were grown on vertically oriented MS plates for 4–5 d. Afterwards, 20 plants were transferred to new MS plates lacking or supplemented with 10 μM ABA. The plates were scanned on a flatbed scanner after 10 d to produce image files suitable for quantitative analysis of root growth using the NIH Image software ImageJ

Drought resistance experiments

Three-week-old plants (*n*=10, three independent experiments) were grown under greenhouse conditions (40–50% room humidity) and standard watering for 15 d. Watering was withheld for 20 d [Pd27-overexpressing (OE) lines] or 8 d (PdPP2C79 OE lines), and then restored. Survival of the plants was scored 5 d later.

Infrared thermography

Plants were grown in a controlled-environment growth chamber at 22 °C under a 12 h light, 12 h dark photoperiod at 100 μE m^{-2} s^{-1} and 40–50% room humidity. Philips bulbs were used (TL-D Super 8036 W, white light 840, 4000K light code). Infrared thermography images of rosette leaves were acquired from 6-week-old plants with an FLIR E95 thermal camera equipped with a 42° lens. Images were processed and quantified with the FLIR tools software. For quantification, the average temperature of 15 different sections corresponding to four leaves per plant was calculated. Five plants per genotype were analyzed in each experiment. The mean temperature ±SD of all the plants for each genotype was reported. Statistical comparisons among genotypes were performed by pairwise *t*-tests.

PP2C inhibition assays

The expression in bacteria and purification of 6His-ΔNHAB1, PdPP2C55, PdPP2C79, Pd15, Pd27, Pd32, Pd44, and Pd957 was performed as described by Santiago *et al.* (2009). His-tagged proteins were purified using Ni–NTA affinity chromatography, eluted and analyzed by SDS–PAGE, followed by Instant Blue staining. Phosphatase activity of ΔNHAB1 was measured using pNPP (15 mM) as substrate, 1 μM PP2C, and 2 μM of the indicated receptors. Dephosphorylation of pNPP was monitored with a ViktorX5 reader at 405 nm (Antoni *et al.*, 2012). Phosphatase activity of PdPP2C55 and PdPP2C79 was measured using the RRA(phosphoT)VA peptide as substrate, which has a K_m of 0.5–1 μM for eukaryotic PP2Cs. Assays were performed in a 100 μl reaction volume containing 25 mM Tris–HCl pH 7.5, 10 mM MgCl$_2$, 1 mM DTT, 25 μM peptide substrate, 1 μM PP2C, and 2 μM of the indicated receptors. After incubation for 60 min at 30 °C, the reaction was stopped by addition of 30 μl of molybdate dye (Baykov *et al.*, 1988) and the absorbance was read at 630 nm with a 96-well plate reader.

Native gel electrophoresis

Native red electrophoresis was carried out as described previously (Ruiz-Partida *et al.*, 2018). Protein samples, each containing 8.5 μg of purified protein in sample buffer: 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 5 mM MgCl$_2$, 15% glycerol, and 0.02% Red Ponceau 2S, with 50 mM DTT, were loaded onto 15% polyacrylamide gels prepared in 375 mM Tris–HCl (pH 8.8), 10% glycerol, 0.012% Ponceau Red S. A solution of Tris–glycine (pH 8.8) with or without 0.012% Ponceau Red S was used as the cathode and anode buffer, respectively. Electrophoresis was performed at a constant current of 25 mA for 150 min at 4 °C. Finally, proteins were detected in-gel using standard Coomassie Blue staining.

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and *Solanum lycopersicum* (SlPYL1, Sl8g076960) PYL1-like receptors were also included. The nomenclature is given from the last two or three digits of KEGG database entries for Pd genes: 103711815, 103704693, 103709545, 103714404, 103708857, 103696437, 103717496, 103706891, 103707144, 103702957, 103698527, and 103708332. The tree was constructed using GeneDoc and ClustalW software. (B) Sequence and secondary structure alignment of date palm ABA receptors and Arabidopsis, sweet orange, and tomato PYL1 proteins. The predicted secondary structure of the date palm receptors is indicated, taking as a model the crystallographic structure of AtPYL1 (Protein DataBank Code 3JRS) and using the ESPRIPT program (http://espript.ibcp.fr/ESPript/ESPript/). Boxes indicate the position of the gate and latch loops. Black asterisks mark residues involved in interactions with ABA's carboxylate group (K86, E121, Y147, S149, and E171 of AtPYL1), either direct contact for the Lys side chain or hydrogen bonds mediated by internal water molecules for the other residues.
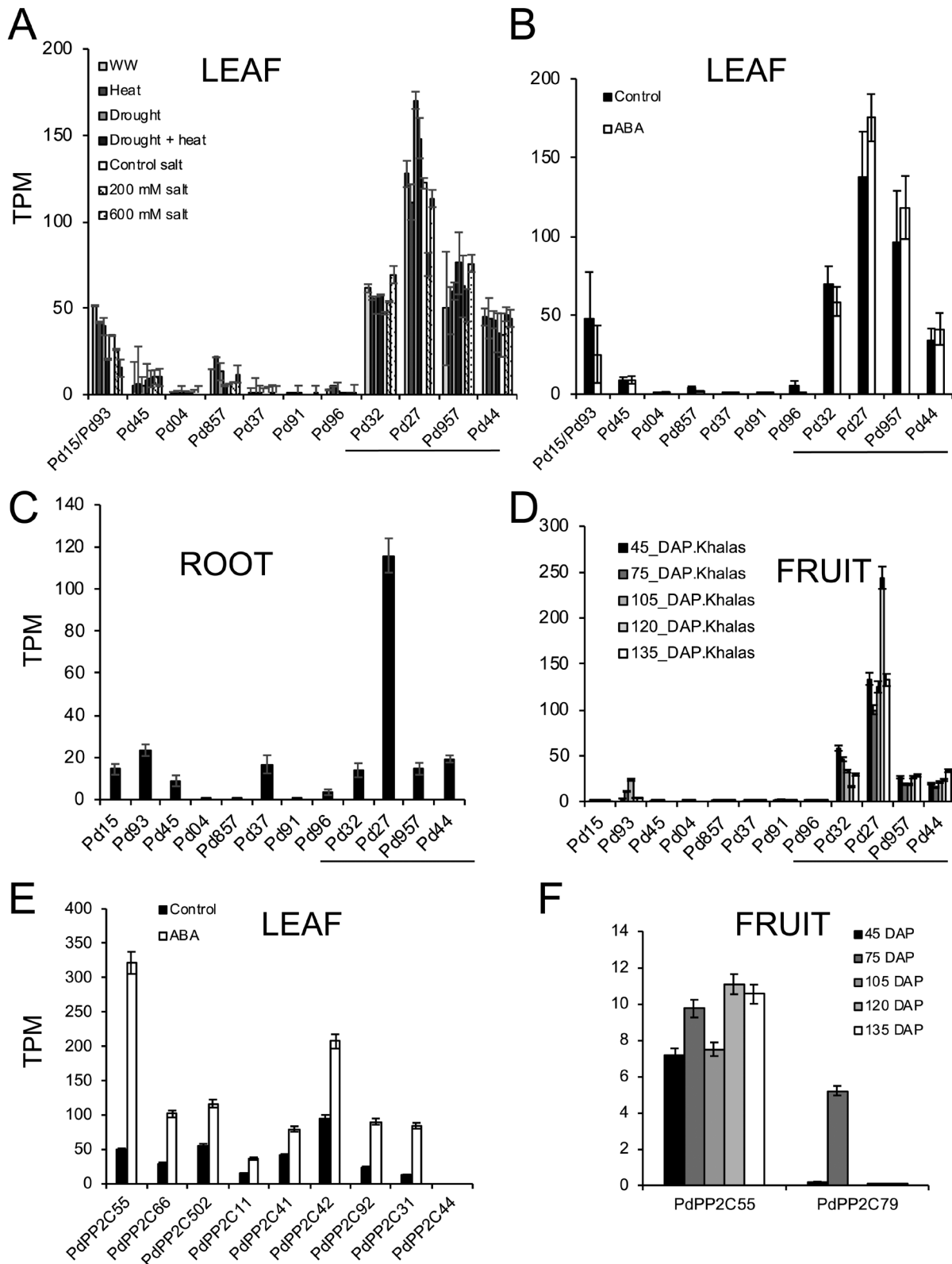
Fig. 2. Relative gene expression of date palm receptors in abiotic stress conditions and different tissues was determined by RNA-seq analysis. PYL8-like receptors are underlined. TPM (transcripts per million) normalizes transcript expression according to transcript length and sequencing depth. (A) Expression of Pd ABA receptors in leaves under well-watered (WW), heat, drought, heat+drought, or salt treatment. (B) Expression of Pd ABA receptors

Results

The date palm genome encodes 12 putative PYR/PYL/ RCAR ABA receptors

We performed a BLAST search in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg-bin/show_organism?org=pda) to identify PYR/PYL/RCAR Pd ABA receptors using as a query the Arabidopsis receptors PYL1, PYL4, and PYL8. As a result, we found a family composed of 12 Pd ABA receptors (Fig. 1A). With the exception of Pd91, they were distributed in three subfamilies, which match the corresponding groups from Arabidopsis receptors. This enables a tentative translation to Pd ABA receptors of those biochemical and physiological features that are already known in Arabidopsis ABA receptors (Rodriguez et al., 2019). In subfamily III, we found three Pd receptors that show sequence similarity to dimeric AtPYL1, whereas in both subfamilies II and I we found four Pd receptors that are related to PYL4 and PYL8, respectively. Given that the Arabidopsis genome contains 14 receptors and date palm only 12, it is not possible to establish a direct correlation in the nomenclature with each Arabidopsis receptor. Therefore, we have preferred to maintain the KEGG–NCBI–Gene ID nomenclature to assign unambiguous names, using the last two or three digits of the full KEGG code to name each individual receptor (Fig. 1). Tentatively, some features of Arabidopsis PYL receptors can be assigned to Pd receptors based on sequence similarity and their location in different branches of the family. Amino acid sequence alignment of Pd ABA receptors (Fig. 1B) revealed the conserved residues of the gate (SGLPA) and latch (HRL) loops (defined by the b3–b4 and b5–b6 regions), which are a hallmark to define PYR/PYL/RCAR ABA receptors in different plant species (Rodriguez et al., 2019).

Transcription profiles of Pd ABA receptors reveal primacy of the PYL8-like family in abiotic stress response

Different RNA-seq gene expression data for the Pd transcriptome have been published (Bourgis et al., 2011; Yin et al., 2012; Al-Mssallem et al., 2013; Yaish et al., 2017; Hazzouri et al., 2019; Xiao et al., 2019). We extracted transcription profiles of Pd ABA receptors from abiotic stress studies performed by Müller et al. (2017) and Safronov et al. (2017). Additionally, in this study, we generated new RNA-seq data after mock or salt treatment (200 mM and 600 mM) of 2-year-old date palms. We screened the ArrayExpress archive (E-MTAB-5261) at EMBL-EBI (https://www.ebi.ac.uk/arrayexpress/submit/overview.html)

in order to obtain gene expression profiles for the 12 Pd ABA receptors after different treatments (Fig. 2A). In Arabidopsis, at least some representative members of the PYR1/PYL1-like, PYL4-like, and PYL8-like subfamilies are expressed to similar levels (Gonzalez-Guzman et al., 2012), but few analyses have been reported in crops (Gonzalez-Guzman et al., 2014). Interestingly, in a crop adapted to harsh conditions such as date palm, we found that four PYL8-like and one/two PYL1-like ABA receptors were preferentially expressed, whereas gene expression of the rest was low or almost undetectable in leaves under the following conditions: well-watered, heat, drought, heat+drought, salt, or ABA treatment (Fig. 2A, B). Pd15 and Pd93 are PYL1-like receptors that show high nucleotide sequence identity and some of the 100 bp RNA-seq data were ambiguous reads, therefore we could not unequivocally assign them. Abiotic stress and ABA treatment did not substantially affect transcript expression of ABA receptors (Fig. 2A, B).

Given that date palm has a well-designed and specialized root system to optimally manage water uptake, we investigated expression of Pd ABA receptors in root tissue (Xiao et al., 2019). Expression of several receptors was detected in roots, and the PYL8-like Pd27 receptor in particular was expressed at 5- to 6-fold higher levels than other receptors (Fig. 2C). PYL8 plays an important and non-redundant role for ABA signaling in Arabidopsis root (Antoni et al., 2013; Belda-Palazon et al., 2018). The predominant expression of Pd27 (and other PYL8-like receptors) in root tissue suggests that ABA signaling mediated by PYL8-like receptors might play a key role to maintain primary root growth at low water potential, to regulate root system architecture or for root hydrotropism, as has been described in other plant species (Sharp et al., 2004; Dietrich et al., 2017; Orman-Ligeza et al., 2018). Expression of Pd15 and Pd93 could be distinguished in 150 bp RNA-seq reads of root tissue (Xiao et al., 2019), and also Pd45 or Pd37 were expressed in roots, although to 10–15% levels compared with Pd27. Finally, RNA-seq data were also compiled for different fruit stages (at 45, 75, 105, 120 and 135 DAP) and, again, PYL8-like ABA receptors showed the highest expression, whereas the rest were expressed at very low levels or were undetectable (Fig. 2D).

In vitro activity of Pd ABA receptors against PP2Cs of Arabidopsis and Phoenix dactylifera

Inhibition of clade A PP2Cs by ABA receptors is the key signature of their biochemical function (Ma et al., 2009; Park et al., 2009; Santiago et al., 2009). In order to biochemically characterize Pd ABA receptors, we have obtained recombinant

in mock- or ABA-treated leaves. Data were obtained from the ArrayExpress archive (E-MTAB-5261) at EMBL-EBI. (C) Expression of Pd ABA receptors in root. Data were obtained from the Sequence Read Archive (experiment PRJNA497070) (Xiao et al. 2019). (D) Expression of Pd ABA receptors in fruit. Data were obtained from the Sequence Read Archive (experiment SRX122676) and correspond to different stages of fruit development after pollination: 45, 75, 105, 120, and 135 DAP (Hazzouri et al., 2019). (E) Expression of Pd PP2Cs in mock- or ABA-treated leaves. Data were obtained from the ArrayExpress archive (E-MTAB-5261) at EMBL-EBI. (F) Expression of PdPP2C55 and PdPP2C79 in fruit at the indicated DAP (Hazzouri et al., 2019).
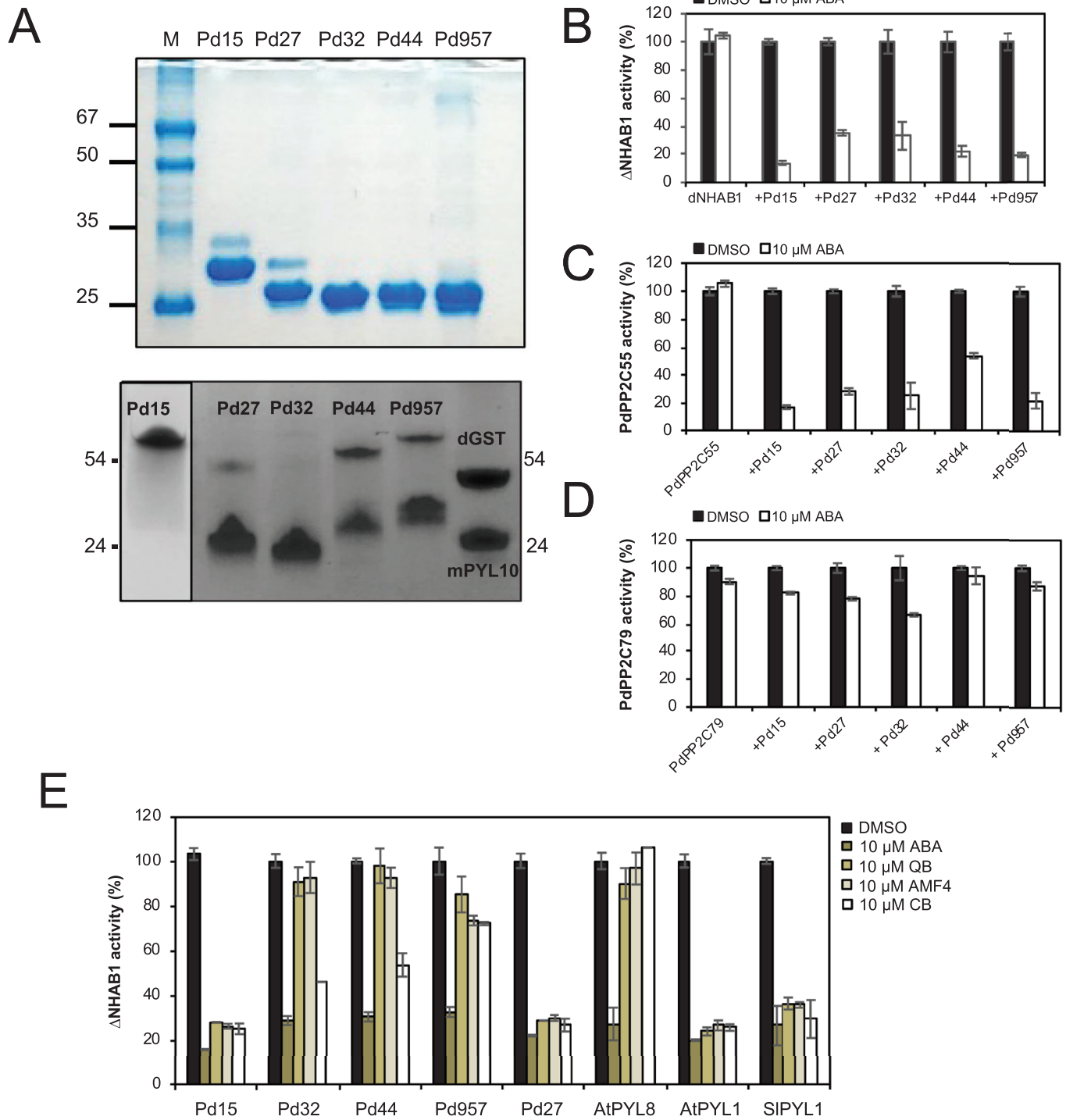
Fig. 3. Expression, purification, and analysis of recombinant Pd ABA receptors. (A) SDS–PAGE (top) and native gel electrophoresis (bottom) of Pd15, Pd27, Pd32, Pd44, and Pd957 ABA receptors. Molecular mass markers in the SDS–PAGE are indicated in kDa, whereas in the native gel we used monomeric PYL10 and dimeric GST as markers. (B–D) ABA-dependent PP2C inhibition mediated by Pd ABA receptors. PP2C activity was measured *in vitro* using the pNPP substrate (for ΔNHAB1) or the RRA(phosphoT)VA phosphopeptide (for PdPP2C55 and PdPP2C79) in the absence (DMSO) or presence of ABA at a 1:2 ratio (phosphatase:receptor). Data are averages ±SD for three independent experiments. Phosphatase activity in the absence of receptor and ABA was taken as 100% activity. (E) Inhibition of ΔNHAB1 phosphatase activity in the presence of Pd, AtPYL8, AtPYL1, or SlPYL1 receptors mediated by 10 μM ABA or different ABA agonists: cyanabactin (CB), quinabactin (QB), and AMF4.
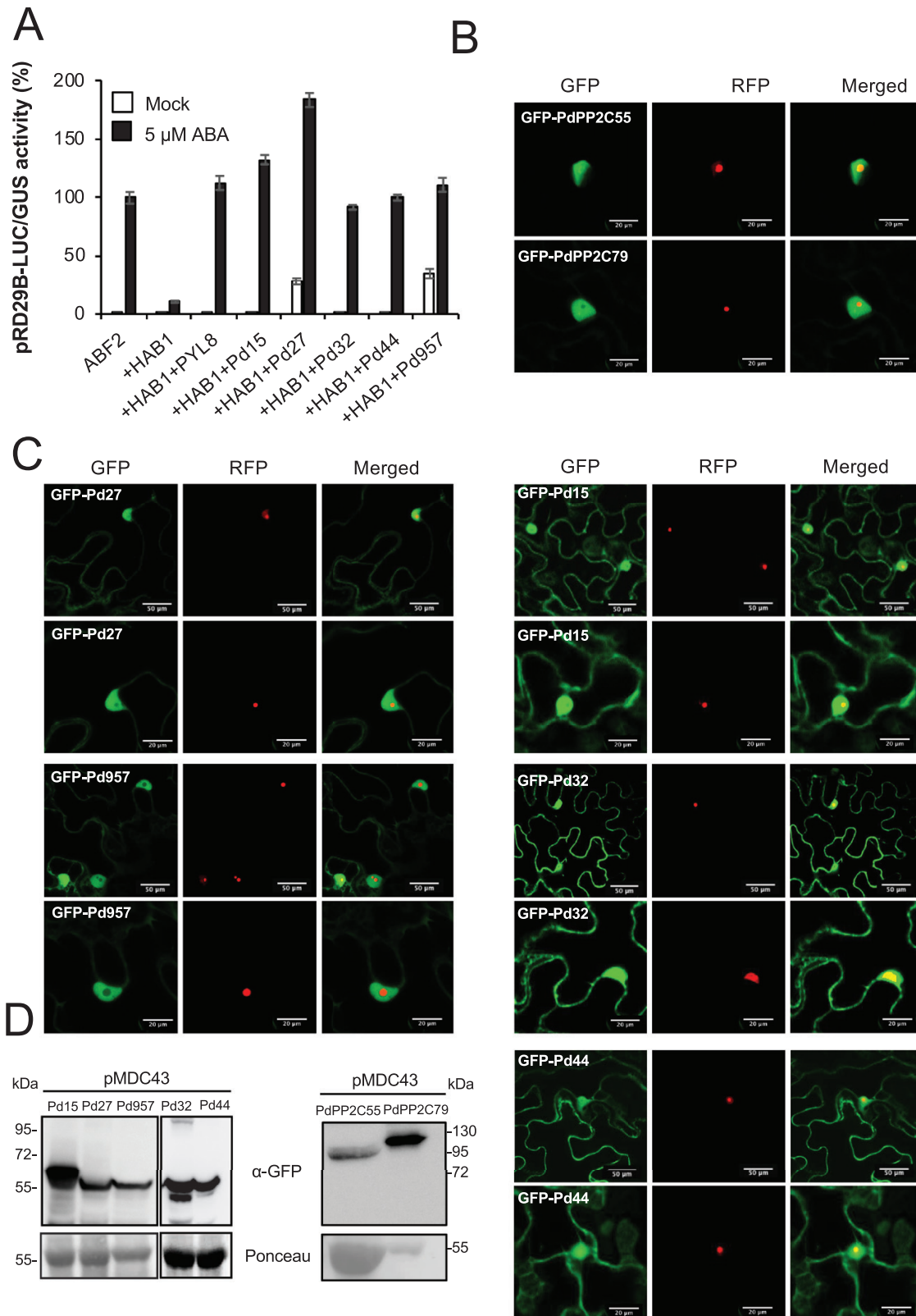
Fig. 4. ABA signaling mediated by Pd ABA receptors in protoplasts and subcellular localization of PdPP2Cs and ABA receptors. (A) Protoplasts of wild-type Col-0 were transfected with plasmids encoding the indicated proteins in the absence or presence of ABA. LUC activity was measured in protein extracts prepared from protoplast suspensions 6 h after transfection, in either the absence or presence of 5 μM exogenous ABA (added 3 h after

protein for five of them. We ordered synthetic genes of Pd15, Pd27, Pd32, Pd44, and Pd957, and cloned them in pCR8 and next in the pETM11 vector for expression in *Escherichia coli*. Recombinant His-tagged protein was obtained for each receptor (Fig. 3A, top) and, in addition to SDS–PAGE, we performed native gel electrophoresis in order to test the oligomeric state of each protein (Fig. 3A, bottom). The PYL1-like Pd15 receptor migrated as a dimer, whereas the PYL8-like Pd27, Pd32, Pd44, and Pd957 receptors migrated mostly as monomers, which shows a good correspondence with the oligomeric state of the Arabidopsis relatives (Dupeux *et al.*, 2011; Hao *et al.*, 2011). Next, we measured the capability of Pd receptors to inhibit Arabidopsis phosphatase HAB1 using pNPP as a substrate. As a result, all Pd ABA receptors showed ABA-dependent inhibition of HAB1 (Fig. 3B).

In order to identify Pd PP2Cs that might be a target of Pd ABA receptors, we performed a BLAST search in the date palm genome using ABI1 as a query to identify closely related Pd PP2Cs (Supplementary Fig. S1). Arabidopsis PP2Cs form a large family composed of 76 members (Schweighofer *et al.*, 2004), and >100 genes in date palm show the PP2C signature (https://www.genome.jp/kegg-bin/show_organism?org=pda). We selected the top 10 PP2Cs that showed the highest sequence similarity to the catalytic PP2C domain of ABI1 as putative clade A PP2Cs from *P. dactylifera* and we used the last two digits of the full KEGG code to name them (Supplementary Fig. S1). Further phylogenetic analysis revealed that some of these PP2Cs showed closer relationship to HAB1, AHG1, or PP2CA (Supplementary Fig. S1) Transcription profiles of PdPP2Cs were obtained from RNA-seq studies performed by Müller *et al.* (2017). ABA treatment strongly up-regulated expression of PdPP2Cs as occurs in Arabidopsis and other plant species (Fig. 2E) (Gonzalez-Guzman *et al.*, 2014; Wang *et al.*, 2019). We ordered synthetic genes for PdPP2C55 (the most expressed PP2C after ABA treatment) and PdPP2C79, which is only expressed during fruit development (Yin *et al.*, 2012; Hazzouri *et al.*, 2019) (Fig. 2F). PP2C79 shows high similarity to the Arabidopsis seed-specific PP2C AHG1, and a closely related PP2C is present in the oil palm (*Elaeis guineensis*) as well as other crops (Supplementary Fig. S2A, B). AHG1 plays an important role in regulating seed dormancy in Arabidopsis via interaction with DOG1; however, AHG1-like PP2Cs in monocot species have not been investigated yet (Née *et al.*, 2017; Nishimura *et al.*, 2018). The corresponding synthetic genes were cloned in the pETM11 vector for expression in *E. coli*, and recombinant His-tagged protein was obtained for

both PP2Cs. PdPP2C55 phosphatase activity was measured using the RRA(phosphoT)VA phosphopeptide as a substrate because pNPP was a poor substrate for this phosphatase in contrast to HAB1. We found that all Pd ABA receptors showed ABA-dependent inhibition of PdPP2C55 (Fig. 3C). Therefore, Pd ABA receptors are functional *in vitro* to inhibit both HAB1 and PdPP2C55 in an ABA-dependent manner.

PdPP2C79, as previously reported for AHG1, lacks a conserved Trp residue required for efficient binding to ABA's ketone group and Arabidopsis ABA receptors (Dupeux *et al.*, 2011) (Supplementary Fig. S2A). In contrast, this Trp residue is conserved in other clade A PP2Cs (Trp385 in HAB1, Trp300 in ABI1) and establishes a water-mediated hydrogen bond network (known as a Trp lock) with the ketone group of ABA and the gate/latch loops of the receptor (Dupeux *et al.*, 2011). Therefore, mutations in this conserved Trp residue (HAB1^{W385A} and ABI1^{W300A}), or changes in neighboring residues as occurs in *Striga hermonthica* PP2C1, render receptor-insensitive PP2Cs that lead to attenuation of ABA signaling (Dupeux *et al.*, 2011; Fujioka *et al.*, 2019). In general, PP2C79 was quite refractory to inhibition by Pd ABA receptors and, in the best case, Pd32 rendered only 30% inhibition of this phosphatase whereas it inhibited PdPP2C55 by 80% at 10 μM ABA (Fig. 3D). Expression of PP2C79 in Arabidopsis led to plants that were strongly insensitive to ABA in germination and root growth assays (Supplementary Fig. S2C, D). Additionally, these plants showed higher water loss than wild-type Col-0 and were prone to wilting when watering was withheld (Supplementary Fig. S2E). Ectopic expression of PdPP2C79 in root tissue led to reduced sensitivity to ABA-mediated inhibition of root growth (Supplementary Fig. S2F). Given that in date palm PdPP2C79 is only expressed during fruit development, these results suggest that PdPP2C79 might provide a mechanism to attenuate ABA signaling at that stage in order to prevent developmental arrest induced by ABA accumulation (Xiao *et al.*, 2019).

Sensitivity of Pd ABA receptors to ABA agonists

ABA receptor agonists are a promising tool to control plant transpiration and drought resistance, and several agonists have been found to be active against Arabidopsis ABA receptors (Helander *et al.*, 2016; Okamoto and Cutler, 2018). However, few examples have been reported of agonist activity against crop ABA receptors (Gonzalez-Guzman *et al.*, 2014; Vaidya *et al.*, 2019). We used Pd receptors as targets to test the effect of several ABA receptor agonists: QB (Okamoto, 2013; Cao *et al.*,

transfection). The activity of the LUC reporter was normalized with GUS activity and the LUC/GUS ratio is indicated. (B and C) Subcellular localization of PdPP2Cs and ABA receptors. PdPP2C55 and PdPP2C79 localize predominantly to the nucleus, whereas Pd ABA receptors localize to the nucleus and cytosol upon transient expression in *N. benthamiana* leaf cells. Confocal images of transiently transformed epidermal cells expressing GFP–PdPP2C55, GFP–PdPP2C79, GFP–Pd15, GFP–Pd27, GFP–Pd32, GFP–Pd44, or GFP–Pd957. The GFP channel shows the subcellular localization of the GFP fusion proteins, whereas the RFP channel shows the nucleolar marker fibrillarin–RFP. Merged indicates the overlap of the GFP and RFP channels. Scale bars correspond to 50 μm or 20 μm. (D) Immunoblotting analysis using anti-GFP antibodies was used to verify the expression of the corresponding fusion proteins.
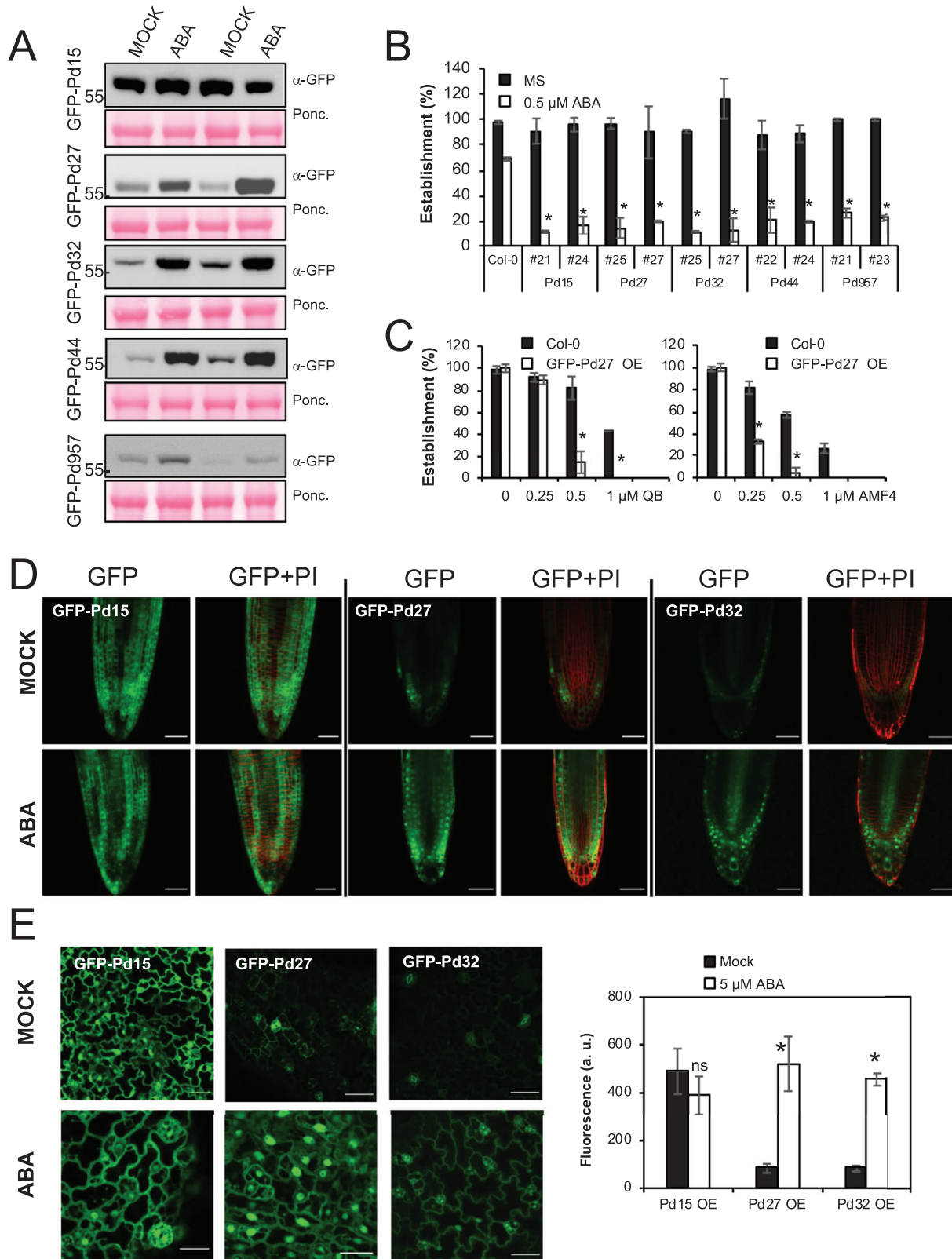
Fig. 5. PdPYL8-like receptors expressed in Arabidopsis accumulate higher levels upon ABA treatment. (A) Immunoblot analysis using anti-GFP antibody shows that ABA treatment enhances protein levels of PdPYL8-like receptors (two independent Arabidopsis T_3 transgenic lines for each palm receptor). Ponceau staining is shown below. (B) GFP–Pd fusion proteins are functional in Arabidopsis. ABA-mediated inhibition of seedling establishment in transgenic lines compared with non-transformed Col-0 plants. The asterisk indicates $P<0.05$ (Student's t-test) when comparing data of transgenic lines

2013), AMF4 (Cao *et al.*, 2017), and cyanabactin (CB) (Vaidya *et al.*, 2017) (Fig. 3E). AtPYL1 and SlPYL1 were sensitive to ABA agonists that target dimeric receptors such as QB, CB, and AMF4, and Pd15 behaved similarly, which further confirms that Pd15 belongs to the PYL1-like branch. Pd32 and Pd44 were resistant to QB and AMF4, but were sensitive to CB, which not only targets the dimeric PYR1 and PYL1 receptors in Arabidopsis, but also inhibits PYL5 efficiently (Vaidya *et al.*, 2017). Pd957 sensitivity to QB, CB, and AMF4 was low (only 20–30% inhibition of phosphatase activity), and AtPYL8 was even less sensitive to these agonists (Fig. 3E). Unexpectedly, Pd27 inhibited by 80% the activity of HAB1 in the presence of 10 μM of any agonist, which suggests that crop receptors can show different sensitivity to ABA agonists compared with Arabidopsis receptors. Vaidya *et al.* (2019) reported that a single amino acid difference between wheat PYL8 and Arabidopsis PYL8 led to a high PP2C inhibitory effect of opabactin with wheat PYL8 and a low effect with AtPYL8. Inspection of wheat subfamily I ligand-binding pockets revealed that the bulky Leu163 of AtPYL8 was replaced by a smaller Val179 in TaPYL8 (Vaidya *et al.*, 2019). The Leu of AtPYL8 would cause a steric clash for the binding of opabactin, whereas it seems that the smaller Val of TaPYL8 avoids it and leads to high agonist potency for PP2C inhibition. Similar minor changes occur in the sequence of Pd27 (or TaPYL8) compared with AtPYL8, for example Leu126 of AtPYL8 (close to the conserved latch loop) is replaced by Val143 in Pd27 and Val142 in TaPYL8 (Fig. 1B). Further structural work with crop receptors is required for a full understanding of agonist sensitivity.

In vivo *activity of Pd ABA receptors*

We tested the *in vivo* activity of Pd ABA receptors by measuring ABA signaling (LUC expression driven by the ABA-responsive RD29B promoter) induced in protoplasts upon ABA perception. To this end, we transfected effector DNA constructs together with an ABA-responsive LUC reporter in the absence or presence of exogenous ABA (Fujii *et al.*, 2009). A constitutively expressed GUS reporter was used for normalization and, accordingly, the LUC/GUS activity ratio was used to measure ABA signaling (Fujii *et al.*, 2009). In the presence of ABA, LUC activity was elicited by the effector ABF2 construct, whereas co-expression of HAB1 abolished ABA-induced LUC activity (Fig. 4A). Introduction of the PYL8 receptor

together with the ABF2+HAB1 expression cassettes restored ABA signaling as did introduction of each Pd ABA receptor. In the case of Pd27 and Pd957, in the absence of exogenous ABA addition, some induction of LUC activity was also observed. In order to visualize the subcellular localization of Pd ABA receptors and PdPP2Cs, we performed transient expression in *N. benthamiana*. In addition to GFP fusion proteins of receptors or phosphatases, we also co-expressed the nucleolar marker fibrillarin–red fluorescent protein (RFP) (Belda–Palazon *et al.*, 2019). In the case of PdPP2C55 and PdPP2C79, both were predominantly localized in the nucleus of *N. benthamiana* epidermal leaf cells (Fig. 4B). We found that Pd ABA receptors were localized in both the nucleus and cytosol; however, Pd27 and Pd957 showed preferential nuclear localization (Fig. 4C). Expression of the GFP fusion proteins was verified by immunoblot analysis (Fig. 4D).

Overexpression of monomeric ABA receptors confers enhanced response to ABA and drought resistance (Santiago *et al.*, 2009; Gonzalez-Guzman *et al.*, 2014; Yang *et al.*, 2016; Mega *et al.*, 2019), and tomato ABA receptors are functional when expressed in Arabidopsis (Gonzalez-Guzman *et al.*, 2014). We were interested in testing whether monocot ABA receptors such as date palm receptors were functional in a dicot species such as Arabidopsis. We expressed GFP-tagged Pd ABA receptors in Arabidopsis as a first step towards analyzing their role in ABA signaling. Expression of the proteins in vegetative tissue was analyzed by immunoblot analysis and, since ABA promotes stabilization and accumulation of the Arabidopsis PYL8 receptor (Belda–Palazon *et al.*, 2018), we analyzed protein expression in mock- and ABA-treated plants (Fig. 5A). The Pd15 protein level did not substantially change in mock- and ABA-treated samples, whereas PYL8-like Pd receptors showed ABA-induced accumulation (Fig. 5A). Therefore, this result reveals that ABA-induced accumulation of PYL8-like receptors also occurs when monocot receptors are expressed in Arabidopsis (Belda–Palazon *et al.*, 2018; Fig. 5A). Moreover, analysis of ABA response in transgenic lines overexpressing GFP–Pd receptors revealed enhanced sensitivity to ABA-mediated inhibition of seedling establishment compared with wild-type Col-0, indicating that Pd receptors are functional when expressed in Arabidopsis (Fig. 5B). In agreement with the Pd27 sensitivity to ABA agonists (Fig. 3E), we observed enhanced sensitivity to QB and AMF4 in plants overexpressing GFP–Pd27 compared with wild-type Col-0 (Fig. 5C). Finally, we confirmed

with non-transformed Col-0 plants in the same assay conditions. Seedlings were scored for the presence of both green cotyledons and the first pair of true leaves 7 d after sowing. (C) Enhanced sensitivity to QB and AMF4 of GFP–Pd27 OE plants compared with wild-type Col-0. Approximately 25 seeds of the indicated genotype (three independent experiments) were sown on MS multiwell plates lacking or supplemented with the indicated concentration of QB or AMF4, and seedling establishment was scored for the presence of both green cotyledons and the first pair of true leaves after 7 d. Values are averages ±SD. The asterisk indicates *P*<0.01 (Student's *t*-test) with respect to the wild type assayed in the same conditions. (D and E) GFP–Pd27 and GFP–Pd32 accumulate after ABA treatment, whereas GFP–Pd15 does not. CLSM imaging of Arabidopsis root apex (D) or leaf tissue (E) in lines expressing GFP–Pd15, GFP–Pd27, or GFP–Pd32 that were mock or ABA treated for 1 h. Two independent lines were analyzed, and representative images obtained with one line are shown. Fluorescence was quantified (arbitrary units) in 10 leaves (three independent experiments) of each transgenic line using images acquired by CLSM.
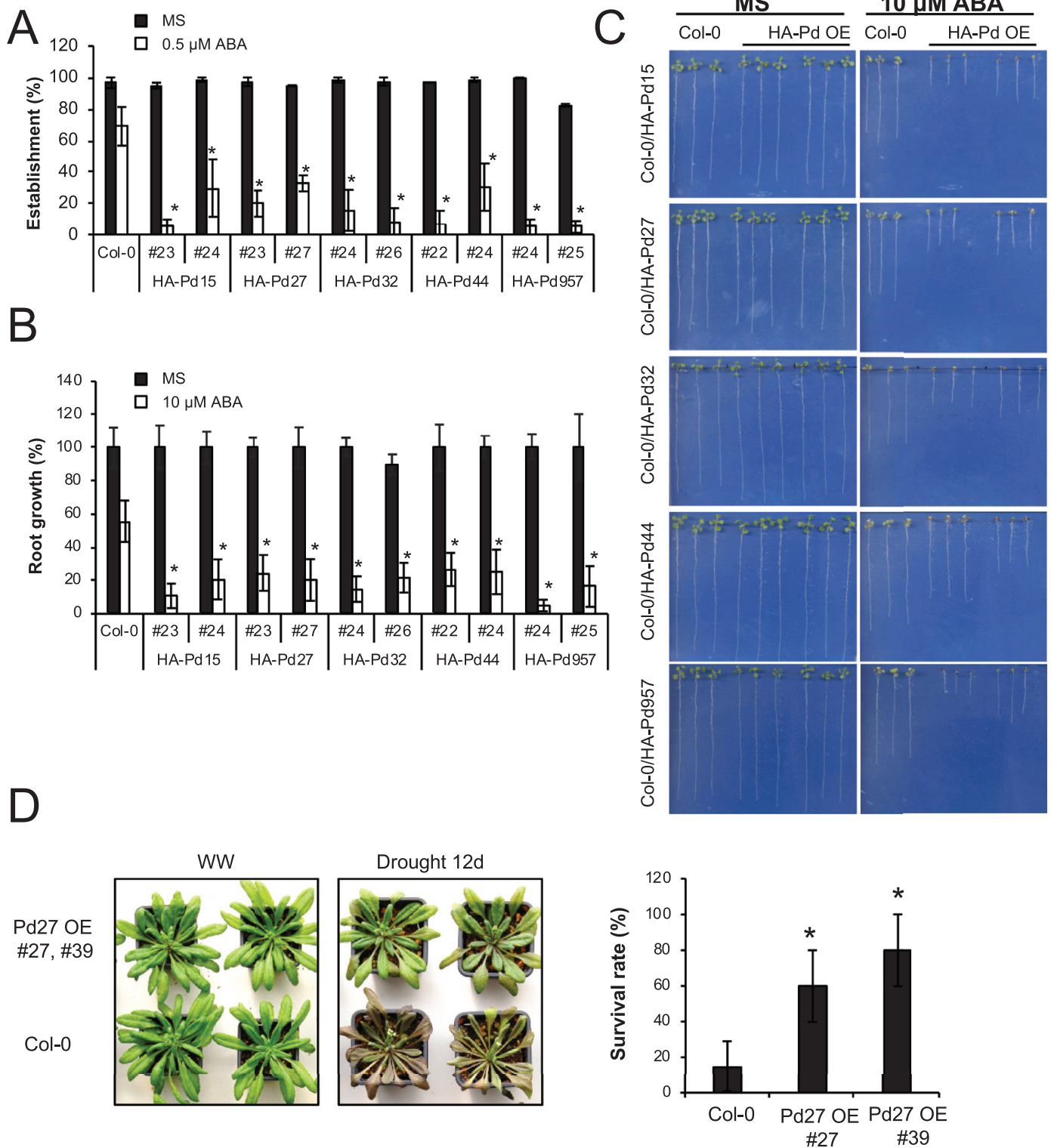
Fig. 6. Enhanced sensitivity to ABA conferred by overexpression of Pd ABA receptors. (A) Enhanced sensitivity to ABA-mediated inhibition of seedling establishment. Approximately 100 seeds of each genotype (three independent experiments) were sown on MS plates lacking or supplemented with 0.5 μM ABA and scored for the presence of both green cotyledons and the first pair of true leaves after 7 d. Values are averages ±SD. The asterisk indicates $P<0.01$ (Student's t-test) with respect to the wild type assayed in the same conditions. (B) Enhanced sensitivity to ABA-mediated inhibition of root growth. Quantification of ABA-mediated inhibition of root growth in the indicated genotypes compared with the wild type. The asterisk indicates

ABA–induced accumulation of the GFP–Pd27 ABA receptor by CLSM imaging, in both root and shoot tissues (Fig. 5D, E). Likewise, GFP–Pd32, another PYL8-like ABA receptor, also accumulates in both tissues in response to ABA, whereas Pd15, which is a PYL1-like ABA receptor, does not accumulate in response to ABA (Fig. 5D, E).

Overexpression of Pd27 confers drought resistance

We also expressed HA–tagged Pd ABA receptors in Arabidopsis and analyzed ABA response of the transgenic lines in seedling establishment and root growth assays (Fig. 6A–C). Like GFP–Pd receptor lines, overexpression of HA–Pd ABA receptors conferred enhanced response to ABA compared with the wild type. In order to test whether palm receptors can confer enhanced drought resistance, we selected transgenic lines overexpressing Pd27 because this receptor is the most expressed in all palm tissues analyzed (Fig. 2). Plants were grown under normal watering conditions for 2 weeks and then irrigation was stopped for 20 d. Non-transformed Col-0 plants wilted, in contrast to transgenic lines that express Pd27 (Fig. 6D). The survival of the plants was scored 5 d after watering was resumed and enhanced survival was found for transgenic plants expressing Pd27 (Fig. 6E). Transpiration was monitored using infrared thermography, and we found that transgenic plants expressing Pd27 showed higher leaf temperature than wild-type Col-0 plants upon spraying with ABA (Fig. 7A). Further physiological analyses were conducted using normalized gravimetric water loss experiments to measure transpiration of wild-type Col-0 and Pd27 OE plants, which was recorded under different soil water potentials, ranging from well-watered (potential near to 0 MPa) to drought conditions (–2.5 MPa) (Fig. 7B). As a result, we found that Pd27 OE plants showed lower transpiration than the wild type. To correlate transpiration and ABA levels, we measured endogenous ABA content (ng g^{-1} DW) by HPLC/MS analysis in the different conditions of soil water potential assayed (Fig. 7C). Interestingly, Pd27 OE plants had a lower ABA content than the wild type, which was particularly evident under drought conditions. Therefore, the combination of transpiration data and ABA content reveals that Pd27 OE plants were more efficient at reducing transpiration at each ABA concentration, particularly when ABA levels increase (Fig. 7D).

Discussion

Abiotic stress is challenging plant agriculture, and ABA is a key phytohormone to trigger plant adaptation to stressors,

particularly under drought conditions (Yang *et al.*, 2019). Date palm is very well adapted to heat and drought conditions, but little is known about the underlying molecular mechanisms (Arab *et al.*, 2016). Guard cell ABA signaling mediated by PdOST1 limits transpiration water loss in date palm, presumably through ABA-dependent activation mediated by ABA receptors (Müller *et al.*, 2017). Recent findings have revealed the role of certain Arabidopsis receptors in stomatal closure signal integration, and, for instance, PYL2 is critical for guard cell ABA-induced responses whereas PYL4 and PYL5 are essential in the responses to CO_2 (Dittrich *et al.*, 2019). In this work, we have identified which members of the date palm family of ABA receptors are particularly relevant for abiotic stress response. The phylogenetic analysis revealed that PdPYLs can be divided into three subfamilies, which correspond to PYL1-like, PYL4-like, and PYL8-like Arabidopsis relatives (Fig. 1). Based on RNA–seq analyses of PdPYLs, and somehow unexpectedly, we discovered that PYL8-like receptors play a major role in the palm response to abiotic stress. This result contrasts with Arabidopsis ABA receptors, where members of the three subfamilies are significantly expressed in different tissues and growth conditions, and Arabidopsis dimeric receptors in particular play an important role to regulate stomatal aperture and adaptation to drought (Gonzalez-Guzman *et al.*, 2012; Okamoto *et al.*, 2013; Dittrich *et al.*, 2019). In contrast to Arabidopsis, four date palm PYL8-like ABA receptors were predominantly expressed under heat, salt, drought, or osmotic stress conditions (Fig. 2). In Arabidopsis, ABA treatment induced their accumulation, which was only previously observed for AtPYL8; therefore, it seems that date palm has expanded this adaptive mechanism to a full branch of ABA receptors for efficient response to abiotic stress.

Specifically, Pd27 was the most expressed receptor in all the conditions analyzed, including root and fruit tissues. Analysis of transgenic palms is not feasible; therefore, we investigated Pd27 in Arabidopsis lines. We measured the effect of Pd27 on transpiration under different soil water potentials, which range from well-watered to drought conditions. In all the conditions tested, expression of Pd27 led to lower transpiration than in the wild type, and interestingly this effect was accompanied by a reduction in ABA levels, particularly at negative soil water potentials (Fig. 7B, C). As a result, reduction of transpiration in Pd27 OE plants per ng of ABA was more efficient than in the wild type (Fig. 7D). Drought leads to a 10- to 40-fold increase of ABA levels (Cutler *et al.*, 2010); therefore, we suggest that stabilization of Pd27 (and probably other PYL8-like receptors such as Pd32) induced by the hormone increase might boost ABA signaling, because higher levels of the receptor will accumulate when ABA levels increase. Transcriptional and

P<0.01 (Student's *t*-test) with respect to the wild type assayed in the same conditions. (C) The photographs show representative seedlings 10 d after the transfer of 4-day-old seedlings to MS plates lacking or supplemented with 10 μM ABA. (D) Enhanced drought resistance of Pd27 OE plants compared with Col-0. Irrigation was withdrawn in 3-week-old plants for 20 d and, after rewatering, survival of the plants was scored after 5 d. Data are means of three independent experiments ±SD (*n*=10 per experiment). The survival percentage 5 d after rewatering is indicated in histograms.
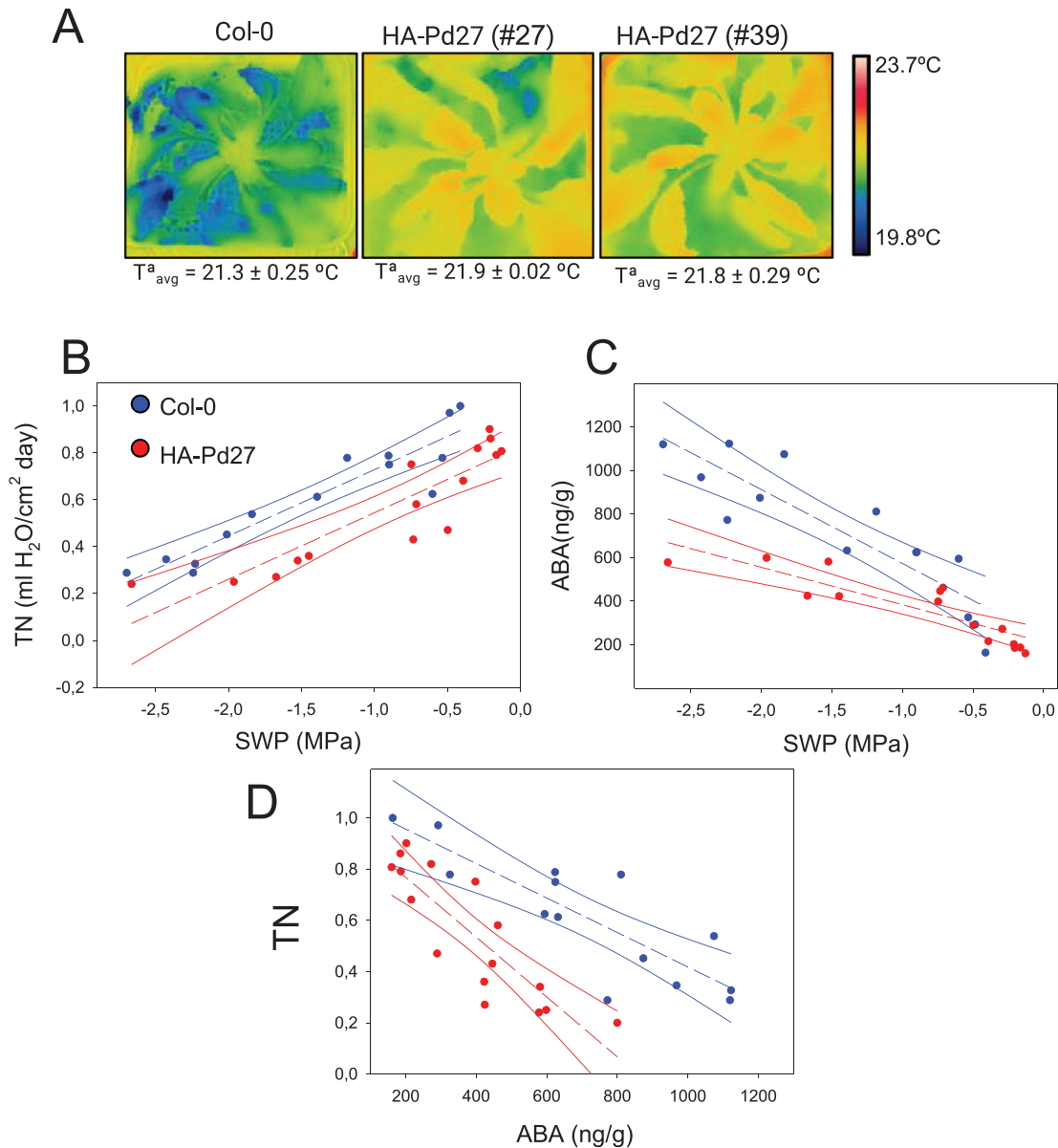
Fig. 7. Plants overexpressing Pd27 show lower transpiration than the wild type. (A) Infrared thermography analysis shows higher leaf temperature of two independent Arabidopsis transgenic lines overexpressing Pd27. False-color images of wild-type Col-0 and Pd27 OE plants (lines #27 and #39) representing leaf temperature, which was quantified by infrared thermal imaging. Data are means ±SD (n=5, ~1000 measurements of square pixels from multiple leaves of each plant). (B) Transpiration assay under different soil water potential (SWP) conditions. (C) ABA quantification under different SWP conditions. (D) ABA lowers transpiration more efficiently in plants overexpressing Pd27 than in the wild type. For experiments of (B–D), n=16. In (B–D), differences in transpiration (TN), endogenous ABA, and transpiration (TN), respectively, were significant due to genotype (P=0.001, P=0.003, and P=0.001, respectively) after ANOVA using SWP (B, C) and ABA (D) as covariant.

post-translational mechanisms limit accumulation of ABA receptors in Arabidopsis (Fernandez *et al.*, 2020); but transcriptional down-regulation either by ABA or by different stressors was not observed in palm receptors. Moreover, ABA treatment in Arabidopsis transgenic lines led to higher accumulation of palm receptors. If this feature of the PYL8-like family is conserved in other crops adapted to drought, it might represent a valuable trait to be introduced in crops lacking such adaptation. Pd27 enhanced drought tolerance upon expression in

Arabidopsis and was able to bind different ABA agonists, whose application together with Pd27 overexpression might enhance plant response to ABA even further. The result of combining Pd27 OE plants with spraying of different agonists when stress occurs might represent a powerful tool for tuning water use dynamically (Cao *et al.*, 2017; Dejonghe *et al.*, 2018; Vaidya *et al.*, 2019). Additionally, the chemical approach benefits from higher expression of the ABA receptor because a lower dosage of the chemical is needed.

This study also reports the ABA- and receptor-resistant activity of the phosphatase PdPP2C79, as well as the extended presence of AHG1-like PP2Cs in different crops, both monocot and dicot species. Given that AHG1 is a target of a key quantitative trait locus that regulates seed dormancy—Arabidopsis DOG1 (Née *et al.*, 2017; Nishimura *et al.*, 2018)—these results suggest that AHG1–DOG1 interaction might be evolutionarily conserved and deserves further research in crops. Additionally, loss of the Trp lock in AHG1-like PP2Cs seems to have markedly reduced their regulation by ABA and ABA receptors, which presumably enables AHG1 activity at plant developmental stages where high ABA concentration accumulates.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Phylogenetic analysis reveals the presence of HAB1-like, AHG1-like, and PP2CA-like PP2Cs in date palm.

Fig. S2. AHG1-like PP2Cs lacking the Trp lock residue are present in both monocot and dicot plant species.

Fig. S3. Expression of PdPP2C79 in Arabidopsis reduces ABA sensitivity.

Table S1. List of antibodies and oligonucleotides used in this work.

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Author contributions

All authors designed and supervised experiments; IGM, AC, JL-J, MM, CO, JJ, RR-P, GP, and BB-P performed experimental analyses; PLR devised the project and wrote the manuscript with contributions of CO and MM; and all authors analyzed the data and approved the final text.

Data availability

All data supporting the findings of this study are available within the article and its supplementary data published online. RNA-seq crude data are available in the Sequence Read Archive.

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