Pectin acetylesterase 8 influences pectin acetylation in the seed coat, seed imbibition, and dormancy in common bean (Phaseolus vulgaris L.)

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Abstract
The results of a prior transcript profiling study identified a large difference in transcript accumulation of a pectin acetylesterase gene, designated as PAE8, in two genetically related lines of common bean (Phaseolus vulgaris). The results of reverse transcription-quantitative PCR experiments confirmed this difference and revealed that the gene is expressed specifically in the seed coat of developing seeds. Genomic sequence data identified a non-functional allele, due to a five-base pair insertion resulting in a frameshift and premature stop codon. The non-functional pae8 allele was associated with a lack of detectable protein as determined by Western blot. PAE8 accounted for approximately 65% of total pectin acetylesterase activity in the developing seed coat. Lack of functional PAE8 resulted in an approximately 2.5-fold increase in acetylation of soluble pectin in the mature seed coat. The presence of a non-functional pae8 allele was associated with an increased rate of water absorption by the seed and increased percentage of germination in aged seeds. The data suggest that the decreased acetylation of pectin leads to enhanced interaction with Ca2+, contributing to water impermeability.

KEYWORDS
common bean, pectin acetylation, pectin acetylesterase, seed coat, seed dormancy, seed germination, seed imbibition

1 | INTRODUCTION

Common bean (dry bean, Phaseolus vulgaris) constitutes an important source of protein and dietary fiber in human diets (de Ron et al., 2015). Among major pulse crops, common bean is characterized by having a high concentration of soluble dietary fiber (Chen et al., 2016). Pectin is a major component of soluble dietary fiber in legumes, abundant in the seed coat. Pectic substances are a constituent of the cell wall representing a mixture of heterogeneous, highly branched and hydrated polysaccharides rich in D-galacturonic acid (Carpita et al., 2015). They are present in the middle lamella adjacent to the primary walls of neighboring cells. In the seed coat of common bean, pectin is found in the palisade layer, where it forms an impermeable barrier to seed hydration (Esau, 1977). Pectin is synthesized as a highly esterified polymer in the Golgi apparatus, which increases its solubility. O-Acetylation occurs primarily at the C-2 and C-3 positions of D-galacturonic acid in homogalacturonan. Upon secretion, it can be de-esterified by methyltransferases and

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acetylesterases. De-esterification favors interactions with cations like calcium and boron, which is associated with water impermeability.

There is strong experimental evidence linking pectin with an important environmentally induced negative attribute of common bean called the hard-to-cook defect. The “phytase-phytate-pectin” hypothesis explains this defect by stating that seeds stored at high temperature and humidity have increased phytase activity, leading to the release of Ca$^{2+}$ ions which bind carboxyl groups in pectin, forming insoluble pectate complexes in the middle lamella of the cell wall, thus interfering with seed water uptake (Galiotou-Panayotou et al., 2008). Beans carrying the low phytic acid 1 (lpa1) mutation display an approximately 90% reduction in phytate levels (Panzeri et al., 2011). This mutation was originally selected to increase the bioavailability of iron. The presence of the lpa1 allele increased cooking time in a series of different genetic backgrounds (Cominelli et al., 2020). In combination, the lpa1 allele and the lectin leucoagglutinating phytohemagglutinin (PHA-L) resulted in decreased lectin hydrolysis during cooking as compared to wild type. This reduced lectin digestibility was associated with an increased seed Ca$^{2+}$ concentration and enhanced localization of Ca$^{2+}$ to cell walls. These results strongly suggest that the increased cooking time conferred by lpa1 was due to increased interaction between pectin and Ca$^{2+}$.

The establishment of water impermeability of the seed coat acts as a mechanism of physical dormancy, which predominates over physiological dormancy in legumes (Finch-Savage & Leubner-Metzger, 2006). However, the decreased water absorption can result in a decreased rate of germination, or uneven germination which is not desirable for crop production (Smykal et al., 2014). Hence, dormancy was selected against during common bean domestication (Koinange et al., 1996).

Results of a prior transcript profiling study identified a large difference in expression of a pectin acetylesterase gene, PAE8 (de Souza et al., 2014), between closely related genotypes differing in seed protein composition and sulfur amino acid profiles (Liao et al., 2012). The present study reports on the molecular, genetic, and biochemical characterization of PAE8. PAE8 constitutes the major pectin acetylesterase of the seed coat. The large difference in expression is conferred by a naturally occurring loss-of-function mutation, resulting in a premature translational termination. The presence of the loss-of-function allele is associated with an increased rate of seed imbibition and seed germination, which is influenced by the age of the seed.

2 | MATERIALS AND METHODS

2.1 | Sequence analysis

The deduced amino acid sequence of PAE8 (Phytozone accession number, Phvul.003g277600.1) was retrieved from the Phytozone database (Goodstein et al., 2011; Schmutz et al., 2014). Protein processing and subcellular localization were predicted with WoLF PSORT (Horton et al., 2007) and SignalP 4.0 (Petersen et al., 2011). To analyze polymorphisms, gene sequences were aligned with MUSCLE (Madeira et al., 2019). BLASTP 2.3.0 (Altschul et al., 1997; Schäffer et al., 2001) was performed to retrieve and compare sequences of other pectin acetylesterase family members in P. vulgaris. Heat map was generated with MATLAB.

2.2 | Plant material

Seeds of common bean (P. vulgaris L.) were planted in Pro Mix BX Mycorrhizae (Premier Tech, Rivière-du-Loup, Québec). Plants were grown in a growth cabinet (Environmental Growth Chambers, Chagrin Falls, OH) or greenhouse at 23/18°C under a 16 h light/8 h dark cycle. G12882 was grown at 28/24°C under an 11 h light/13 h dark cycle. Phaseolus coccineus was hand pollinated according to Chen et al. (2020). Designation of developmental stages of the seed is according to Walbot et al. (1972).

2.3 | RNA extraction and cDNA synthesis

RNA was extracted using a modified LiCl precipitation method (Bruneau et al., 2006). RNA was quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Mississauga, ON). RNA quality was evaluated from its $A_{260/280}$ ratio and by agarose gel electrophoresis. Total RNA was treated with amplification grade DNase I (Thermo Fisher Scientific). First strand cDNA synthesis was performed with qScript cDNA SuperMix (Quanta Biosciences, Beverly, MA).

2.4 | Quantitative PCR

Gene specific primers were used for PAE8, qPvPAE-704F, 5’-TGCC CACATCTTGCATTCGA-3’ and qPvPAE-962R, 5’-ACCAACACTC ACCGCCTTCTCG-3’, and ubiquitin (UBQ, Phvul.007G052600.1) (Duwadi et al., 2018). Primer efficiency was determined through a standard curve of $C_q$ versus log starting template quantity and assessed as acceptable with efficiency between 95% and 105%. Specificity was confirmed through melt peak analysis and agarose gel electrophoresis. Quantitative PCR was performed with a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, Mississauga, ON). Reactions were carried out in Hard-Shell 96-well PCR plates (Bio-Rad Laboratories) in a final volume of 15 μl. The reaction contained 9 μl of SsoFast EvaGreen Supermix (Bio-Rad Laboratories) with primers at a concentration of 0.5 μM and 6 μl of a 32-fold dilution of the cDNA. The PCR program consisted of an initial step of 3 min at 95°C followed by 40 cycles of 10 s at 95°C and 30 s at 60°C. Data were analyzed using CFX Manager. Data were expressed as the cycle number necessary to reach a threshold fluorescence value ($C_q$). The reported values are the means of three biological replicates consisting of independent RNA extracts; with each biological replicate the average of three technical replicates. Data were normalized to the mean $C_q$ of the reference gene, for which the variation between genotypes was less than or equal to 0.1. Controls without template were
performed in triplicate for each primer pair and displayed no amplification.

2.5 | Immunoblotting

Rabbit polyclonal antibodies were raised against the following peptide at the C-terminal end of PAE8, NH2-C386CQNTVSDLKDHPG1-COOH by Biomatik (Cambridge, ON). Total or soluble protein was extracted from developing seed coats. Tissue was ground in liquid nitrogen. For total protein, tissue was homogenized in extraction buffer (4% sodium dodecyl sulfate, 50 mM Tris-HCl pH 8.0, 50 mM KCl, 1 mM CaCl2, 10% glycerol) containing complete™, EDTA-free Protease Inhibitor Cocktail (Roche Diagnostics, Dorval, QC) and 1 mM dithiothreitol. The extract was centrifuged for 20 min at 13,500×g and the supernatant recovered. For soluble protein, tissue was homogenized in ice-cold extraction buffer (50 mM Tris-HCl pH 8.0, 50 mM KCl, 1 mM CaCl2, 10% glycerol) containing cOmplete™, EDTA-free Protease Inhibitor Cocktail (Roche Diagnostics, Dorval, QC) and 1 mM dithiothreitol. The extract was centrifuged for 20 min at 13,500×g and the supernatant recovered. Protein concentration was measured using the Bio-Rad Protein Assay Reagent. Thirty micrograms of protein was separated on a 10% SDS polyacrylamide gel. Protein was transferred to a PROTRAN® nitrocellulose membrane (Millipore Sigma, Oakville, ON) using a semi-dry transfer apparatus (Bio-Rad Laboratories). PAE8 was detected with anti-PAE8 polyclonal antibodies (1:1,000 dilution) and IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody (Rockland Immunochemicals, Gilbertsville, PA). Signal was captured with an Odyssey Imaging System (LI-COR Biosciences, Lincoln, NB).

2.6 | Assay of pectin acetyl esterase activity

The assay was performed using 100 μg of soluble protein in 100 mM sodium potassium phosphate buffer pH 7 using 2 μM 4-p-nitrophenyl acetate (Bordenave et al., 1995). Product formation was measured at 405 nM using a PowerWave XS Microplate Spectrophotometer (Agilent Technologies, Mississauga, ON).

2.7 | Quantification of pectin acetylation

Seed coats were removed with a hammer and ground in a Kleco ball mill (Visalia, CA). Water-soluble pectin was extracted as described by Gou et al. (2012), with modifications. Two hundred milligrams of flour was extracted in 10 ml of water adjusted to pH 1.5 with HCl and the solution heated at 80°C while agitating at 750 rpm for 1 h. The solution was passed through a 0.45 μm Acrodisc® Syringe Filter with Supor® Membrane (Pall, Mississauga, ON). Pectin was precipitated overnight with two volumes of 95% (v/v) ethanol at 4°C. The pellet was recovered after centrifugation at 3,220×g for 10 min and washed three times with 95% (v/v) ethanol. Samples were air dried. Dried samples were crushed with a mortar and pestle. The samples were resuspended in 1 N NaOH at a concentration of 6 mg/ml. Acetates were released by incubating for 1 h at 26°C with agitation at 750 rpm. The solution was neutralized with HCl. Reactions were centrifuged at 13,500×g for 10 min, and supernatants used to measure acetate content with the Acetate Acid analyzer format test kit (K-ACETAK) from Megazyme (Bray Wicklow, Ireland) according to the user’s manual.

2.8 | Genomic DNA extraction and genotyping

Leaf tissue was frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. Genomic DNA was isolated using the GenElute™ Plant Genomic DNA Miniprep Kit (Millipore Sigma) following the manufacturer’s protocol. PCR was carried out using 50 ng of genomic DNA as template for 35 cycles with Taq DNA polymerase and the following gene specific primers binding a sequence in the proximal promoter: SM-642-664_F1, 5’-AGACCTGAGAACACATCTTTC_3’ and SA-SM-849-872_R1, 5’- GTGACTGGATACATCTCCTAC-3’. The rate of imbibition was assessed for 10 g of dry seeds of each genotype. Seeds were put in a Petri dish (10 cm of diameter), and 20 ml of ultrapure water was added. Seed samples were incubated at room temperature. Each sample was removed from the plate at intervals of 0.5, 1.5, 3.5, and 6 h. The surface water was removed with blotting paper, and soaked seeds were weighed. After weights were recorded, seeds were put back into water and incubation continued. To evaluate germination, seeds were surface sterilized with 0.5% sodium hypochlorite, washed three times with MilliQ water, and placed between two wet gauze pads in a Petri dish. Germination percentage was evaluated after 3 days.

3 | RESULTS

3.1 | A loss-of-function allele is associated with reduced PAE8 expression

In a prior transcript profiling study (Liao et al., 2012), two related genotypes, SARC1 and SMARC1-PN1, differing in seed protein composition, were found to be contrasted in their expression of the pectin acetyl esterase gene, PAE8. Transcript levels of two contigs, both corresponding to PAE8, differed at four seed developmental stages, expression being higher in SMARC1-PN1 than SARC1 by 5- to 16-fold (Table S1). Results from a quantitative RT-PCR experiment indicated that transcript levels of PAE8 are higher in the seed coat than in cotyledon in developing seeds at stage V—cotyledon (50 mg seed weight), by approximately 1,500-fold (Table 1). The results confirmed as well the genotypic difference in expression, with approximately 14-fold higher transcript levels in the seed coat of SMARC1-PN1 as compared with SARC1. PAE8 belongs to a family
of 11 pectin acetyl esterase genes in the *P. vulgaris* genome. Data from the RNAseq gene atlas indicated that PAE8 is the predominant member of this family expressed in developing seeds (O’Rourke et al., 2014) (Figure 1a). To visualize the expression of the PAE8 protein, specific polyclonal antibodies were used in immunoblotting. The PAE8 protein was readily detected in total and soluble protein extracts from seed coat of developing seeds at stage VI—maturation (100–200 mg seed weight), as a protein band of approximately 41 kDa, which matches the predicted molecular weight of the mature enzyme (Figure 1b). Signal was detected exclusively in SMARC1-PN1.

The PAE8 gene is located on chromosome 3, spanning positions 51,454,061 to 51,458,474 and has 13 exons and 12 introns. **PAE8** encodes a protein of 399 amino acid residues with a central pectin acetyl esterase domain. A signal peptide is predicted to be cleaved between positions 24 and 25 to release the mature enzyme in the extracellular space. To analyze putative polymorphisms present in **PAE8**, gene sequences were aligned from SARC1, SMARC1-PN1, and their related germplasm line, SMARC1-PN1, Sanilac, their recurrent parent (Pandurangan et al., 2016), G19833 (Schmutz et al., 2014), BAT93 (Vlasova et al., 2016), and OAC-Rex (Genbank assembly...
accession: GCA_015708805.1). The reference cDNA sequence was included in the alignment to visualize exon-intron boundaries. The alignment revealed an insertion of five nucleotides, 5'-TACTA-3', in the 7th exon in SARC1 and Sanilac, between positions 2,456 and 2,457 of the reference gene sequence. The insertion creates a frameshift introducing a premature stop codon, which produces a truncated protein of 198 amino acid residues.

3.2 | Pectin acetyl esterase activity and pectin acetylation in the seed coat

To determine whether the absence of functional PAE8 protein affects total pectin acetyl esterase activity in the seed coat, enzymatic activity was assayed in vitro in soluble protein extracts with the artificial substrate 4-μ nitrophenyl acetate. Figure 1c shows a plot of the product formed per mg of protein in the assay in function of time. Both curves were fitted to polynomial equations. The value of the initial rate of the reactions is found in the first x term (Leskovac, 2003). The ratio of these terms indicates that pectin acetyl esterase activity is approximately 2.8-fold higher in SMARC1-PN1 than in SARC1. The initial velocity measured in the SMARC1-PN1 extract was relatively high (3.44 μkatal/mg) and comparable to the specific activity reported in cell wall extracts of Vigna radiata hypocotyls (Bordenave et al., 1995).

To determine whether the lack of PAE8 impacts pectin acetylation in the seed coat, water-soluble pectin was extracted from seed coats of mature seeds, and acetate was quantified after saponification. The acetate content in SMARC1-PN1, expressed in μg per mg pectin, was only 40% of the value measured in SARC1 (t test p value ≤0.01) (Figure 1d).

3.3 | Genetic inheritance of PAE8 alleles

Experiments were conducted to understand the inheritance of PAE8 alleles in SARC1 and SMARC1-PN1. Sequence analysis results indicated that the loss-of-function pae8 allele in SARC1 originated from the parental genotype Sanilac. Immunoblotting was performed with SARC1, SMARC1-PN1, and their parental genotypes (Osborn et al., 2003). Signal of a comparable intensity to SMARC1-PN1 was detected in Great Northern US 1140 and a phaseolin-deficient P. coccineus accession (Pandurangan et al., 2016) (Figure 2a). As expected, signal was not detected in Sanilac. Signal was very low in the wild P. vulgaris accession G12882. These results suggested that the functional PAE8 allele in SMARC1-PN1 was inherited either from Great Northern US 1140 or from P. coccineus.

In the alignment of PAE8 gene sequences, beside the 5-bp indel polymorphism described earlier, there were additional linked polymorphisms, including a 150-bp indel in the proximal promoter region between SARC1 and SMARC1-PN1. A gene-specific marker was developed based on this polymorphism. PCR amplification in SARC1 and Sanilac generated a 414 bp fragment, as compared with a 230 bp fragment in SMARC1-PN1 (Figure 2b). The size difference was greater than 150 bp due to additional, smaller indels being present between the two genotypes. The same primers were used to analyze the genetic inheritance of the PAE8 allele. Great Northern US 1140 shared a PCR product of similar length as that of SMARC1-PN1. For G12882 and P. coccineus, the length of the PCR product was intermediate, suggesting that shorter deletions are present in these genotypes. Among reference genomes, BAT93 and G19833 have a shorter deletion of 62 bp, whereas OAC-Rex has the same 150 bp deletion as SMARC1-PN1. Overall, these results confirmed that the functional PAE8 allele in SMARC1-PN1 was inherited from Great Northern US 1140. This correlates with the presence of a parental specific single nucleotide polymorphic (SNP) marker, SS 715645573, in SMARC1-PN1, inherited from Great Northern US 1140, located approximately 1.3 Mb upstream of PAE8 (Diapari et al., 2016; Song et al., 2015; Viscarra-Torrico et al., 2021a, 2021b).

3.4 | Seed imbibition and germination

Since pectin de-esterification favors interactions with divalent cations and is associated with water impermeability, the impact of differential pectin acetylation was tested on the rate of seed imbibition. As
expected, a reduction in water absorption was readily apparent after 30 min in SMARC1N-PN1 as compared with SARC1 (Figure 3a). This difference was also manifested by the fact that after 10 min, SARC1 seeds were wrinkled due to imbibition, and SMARC1N-PN1 seeds were smooth (Figure 3c). By 3 h, seeds of both genotypes had soaked water and looked similar. Next, we sought to test additional genotypes contrasted for the PAE8 allele. Sanilac and Mist (Khanal et al., 2016) are the parents of a recombinant inbred population (Farid et al., 2017). The results of PCR genotyping confirmed that Mist possesses the functional PAE8 allele, like SMARC1N-PN1 (Figure S1). Testing the rate of seed imbibition revealed an even greater reduction in water absorption in Mist as compared with Sanilac, less than one third after 30 min, with incomplete recovery by 360 min (Figure 3b).

Like SARC1 and SMARC1N-PN1, Sanilac seeds were wrinkled after 10 min due to imbibition, while Mist seeds were smooth (Figure 3c). However, after 3 h, contrary to SMARC1N-PN1, some of the Mist seeds had not imbibed water. The recombinant inbred population was genotyped to determine whether lines inherited their PAE8 allele from Sanilac or Mist (Figure S1). Selected recombinant inbred lines were tested for the rate of seed imbibition in relation with their genotype at the PAE8 locus. Like their parents, lines homozygous for the loss-of-function pae8 allele exhibited faster imbibition than those having the functional PAE8 allele (Table 2). This was particularly obvious at the first time point of 0.5 h. Heterozygous lines displayed intermediate values. Non-hydrated seeds were observed exclusively among lines heterozygous or homozygous for the functional allele. Since seed imbibition is an important determinant of germination, germination was tested in seeds of SARC1 and SMARC1N-PN1 of a different age. Young seeds of approximately 5 months old had a similar germination percentage (Figure 3d). However, in seeds of 2.5 and 3.5 years old, germination was sharply reduced in SMARC1N-PN1 as compared with SARC1.

**FIGURE 3** Effect of PAE8 alleles on seed imbibition and germination. Time course of water absorption in seeds of (a) SARC1 and SMARC1N-PN1; (b) Sanilac and Mist; (c) water absorption of SARC1 and SMARC1N-PN1 seeds; (d) germination percentage of seeds of different age of SARC1 and SMARC1N-PN1.
The present study comes on the heels of a recent report by Soltani et al. (2021) which identified the same PAE8 allele in a recombinant inbred population derived from parents differing in their rate of seed water uptake and resistance to flooding, PR9920-171 and TARS-H1. In this study, a second candidate gene was present within the identified quantitative trait locus (QTL) interval encoding a cytochrome P450 707A1 involved in the catabolism of abscisic acid (Saito et al., 2004). The corresponding gene influences physiological dormancy in Arabidopsis (Okamoto et al., 2006). The CYP450 707A1 transcript was present at higher levels in TARS-H1 as compared with PR9920-171, by about tenfold (Soltani et al., 2021), suggesting a possible contribution to the differential rate of water absorption between genotypes. The fact that the same PAE8 allele was identified in the present study in a different set of genotypes and linked with a differential rate of seed water absorption confirms that PAE8 is an important determinant of seed imbibition. This does not exclude the contribution of other genes. In black bean, a major QTL related to seed hardness has been identified on chromosome 7, in proximity to the Asper (Asp) gene responsible for seed coat luster (Sandhu et al., 2018). The identification of PAE8 on chromosome 3 is consistent with prior results of genetic mapping of QTL in the same region. This includes QTL related to seed water absorption (Pérez-Vega et al., 2010), washed drain weight of canned beans (Wright & Kelly, 2011) and seed dormancy (Koinange et al., 1996).

More recently, Diaz et al. (2021) identified a QTL in the same region that inversely controls water absorption capacity and cooking time.

Soltani et al. (2021) associated PAE8 with dormancy by showing that wild accessions in an Andean diversity panel have the functional allele, whereas the loss-of-function paed8 allele dominates in cultivated accessions. The present results show that PAE8 negatively affects germination only in older seeds. Assays performed with the Sanilac × Mist population also indicate that PAE8 can be associated with non-hydrating seeds. The intermediate impact on the rate of water absorption observed with heterozygous genotypes suggests a gene dosage effect on this phenotype.

The biochemical characterization performed in this study revealed that the loss-of-function paed8 allele introducing an early stop codon was associated with the absence of detectable PAE8 protein, as determined by immunoblotting. Data on catalytic activity indicated that PAE8 accounts for approximately 65% of total pectin acetyl esterase activity in the seed coat of developing seeds in SMARC1N-PN1. The loss-of-function paed8 allele was associated with a 2.5-fold higher concentration of acetate residues in pectin in the seed coat of mature seed. This difference in pectin composition seems necessary and sufficient to determine the changes observed in water absorption and germination. Based on the available literature, it seems likely that the decreased substitution of pectin leads to enhanced interactions with Ca2+ and increased water impermeability. From a physiological standpoint, Soltani et al. (2021) showed that the fast imbibing genotype has more micro-cracks on the lens surface near the hilum of the seed. These cracks had been previously correlated with water permeability in soybean seed (Ma et al., 2004).

Soltani et al. (2021) indicated that the presence of functional allele of PAE8 is associated with a greater tolerance to flooding. Great Northern US 1140, the source of the PAE8 allele in SMARC1N-PN1 has been grown extensively in the Canadian prairies, an area prone to flooding (Navabi et al., 2014). It is tempting to speculate that the PAE8 allele may have been retained in this cultivar for greater adaptation to its environment. On the other hand, the present results showed the presence of the same allele in Mist, a cultivar grown in Eastern Canada. The presence of this allele could result in decreased or uneven germination, unfavorable for grain production, and may affect cooking time or canning quality, the main seed quality trait evaluated for new bean cultivars. Soltani et al. (2021) predicted that this allele is likely to be selected against during bean breeding.

The present findings raise interesting questions for future investigation. One of them concerns the origin of PAE8 alleles present in cultivated beans, including wild progenitors. A related question concerns the possible role of PAE8 alleles in market classes other than navy and Great Northern, and the distribution of PAE8 alleles in different bean cultivars. It is also worthwhile asking if a similar paed8 allele was selected in other domesticated Phaseolus species. Further, it might be possible that variation in an orthologous gene, or additional genes

### Table 2

| Line | Genotype   | 0.5 hr | 1.5 hr | 3.5 hr | 6 hr | Non-imbibed seeds (%) |
|------|------------|--------|--------|--------|-----|------------------------|
| 68   | paed8      | 6.15 ± 0.30 | 7.27 ± 0.51 | 8.28 ± 0.52 | 8.82 ± 0.37 | - |
| 78   | paed8      | 5.06 ± 0.52 | 7.13 ± 0.19 | 8.53 ± 0.21 | 9.54 ± 0.13 | - |
| 122  | paed8      | 5.24 ± 0.62 | 7.86 ± 0.56 | 9.49 ± 0.17 | 10.2 ± 0.1 | - |
| 5    | paed8/PAE8 | 2.38 ± 0.21 | 5.30 ± 0.07 | 7.27 ± 0.08 | 8.38 ± 0.08 | - |
| 45   | paed8/PAE8 | 3.75 ± 0.47 | 5.07 ± 0.57 | 5.96 ± 0.83 | 6.55 ± 1.18 | 27 ± 6 |
| 61   | paed8/PAE8 | 1.15 ± 0.34 | 3.69 ± 0.43 | 6.71 ± 0.21 | 8.69 ± 0.18 | 8 ± 3 |
| 4    | PAE8       | 1.38 ± 0.14 | 4.92 ± 0.30 | 7.40 ± 0.29 | 8.81 ± 0.25 | 5 ± 4 |
| 20   | PAE8       | 1.74 ± 0.08 | 5.00 ± 0.06 | 7.57 ± 0.18 | 8.55 ± 0.12 | 15 ± 3 |
| 59   | PAE8       | 0.355 ± 0.102 | 2.59 ± 0.21 | 5.74 ± 0.39 | 8.09 ± 0.44 | 10 ± 7 |

Note: Average ± standard deviation; n = 3.
involved in pectin modification, influences seed hydration in pulse crops outside of the genus Phaseolus. In conclusion, PAE8 has been characterized as the major pectin acetyltransferase gene expressed in the developing seed coat of common bean. A naturally occurring loss-of-function allele has been identified, which is associated with an increased rate of water absorption and enhanced germination in older seeds. This discovery is likely to have practical applications linked to both agricultural production and end use of common bean.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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