Theoretical and Applied Genetics
https://doi.org/10.1007/s00122-020-03698-7

ORIGINAL ARTICLE

Toward the introgression of *PvPdh1* for increased resistance to pod shattering in common bean

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Received: 20 February 2020 / Accepted: 29 September 2020

Abstract

Key message **A common bean shattering-resistance allele of PvPdh1 reduces pod twists during dehiscence, shows dominance that varies by phenotyping method, is part of a selective sweep, and can be introgressed using CAPS markers.**

Abstract Some varieties of common bean (*Phaseolus vulgaris* L.) suffer from pod shattering, which can severely reduce yields, especially in arid conditions. The *PvPdh1* locus on chromosome Pv03 has recently been described as a major locus controlling pod shattering in common bean and could be used to mitigate pod shattering in the future. Despite this, the role of a possible second locus on chromosome Pv08 remains unclear and patterns of dominance and epistasis between alleles of these genes have not been resolved. This information will be vital for efficient selection to decrease pod shattering. Further, the genetic diversity around the *PvPdh1* gene has not yet been thoroughly explored, and there are not yet genetic screens that can be used to evaluate pod shattering in segregating populations. Here, we have developed a recombinant inbred population to determine the roles of genes implicated in pod shattering and evaluate the patterns of dominance among the relevant alleles. Our results suggest that a *PvPdh1* allele reduces pod valve twisting, and its dominance varies by phenotyping method. This allele is the only genetic variant that provides environmentally stable and widespread resistance to pod shattering in Middle American common beans grown for grain. Further analyses identified a selective sweep around *PvPdh1* with greater nucleotide diversity in individuals with the ancestral, shattering-susceptible allele. Finally, we developed simple, effective CAPS markers to facilitate the introgression of *PvPdh1* into new varieties of common bean. These genetic resources will be critical for improving the aridity resilience of a major global staple.

Introduction

Reduced pod shattering is an important breeding target in many crops, including common bean (*Phaseolus vulgaris* L.). In the wild, many legumes benefit from seed dispersal mediated by explosive pod dehiscence, known as pod shattering. During the domestication process, the trait has been strongly reduced across most legume taxa (Ogutcen et al. 2018; Di Vittori et al. 2019). Despite this, some market classes of common bean have persistently high levels of pod shattering, leading to reduced yields and a constrained harvest window. This issue is particularly problematic in semiarid environments, which cause pods to become brittle and fracture more easily (Fig. 1).

Common bean is a vital source of protein and micronutrition for hundreds of millions of people globally (Singh 1999; Gepts et al. 2008). The crop was independently domesticated in Middle America and the Andes (Gepts 1988; Kwak and Gepts 2009; Bitocchi et al. 2013; Ariani et al. 2018; Cortinovis et al. 2020), leading to the species' two major domesticated gene pools. These are additionally subdivided into several ecogeographic races, each with a long history of adaptation to specific environmental conditions (Singh et al. 1991; Beebe et al. 2000). In particular, members of the Middle American ecogeographic race Durango are adapted to the semiarid highland environments of northern Mexico and the southwestern USA, whereas the Middle American race

Communicated by Matthew N. Nelson.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03698-7) contains supplementary material, which is available to authorized users.

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Fig. 1 .

Mesoamerica inhabits humid lowland regions of Mexico, Central America and lowland South America. Useful alleles from any major gene pool can readily be moved into others, and crosses between races have major untapped potential for breeders (Singh et al. 1993). Seven independent domestication events occurred in the *Phaseolus* genus, including close relatives of common bean such as Lima bean (*P. lunatus*), runner bean (*P. coccineus*) year bean (*P. dumosus*) and tepary bean (*P. acutifolius*) (Gepts 2012; Bitocchi et al. 2017). An improved genetic understanding of pod shattering in common bean will be useful for improvement of numerous other domesticated legumes that suffer from pod shattering (Ogutcen et al. 2018; Di Vittori 2019).

Several genes are known to influence resistance to pod shattering in common bean (Rau et al. 2019; Parker et al. 2020), and the genes involved vary by gene pool. In the Middle American domesticated beans, the locus *Phaseolus vulgaris Pod dehiscence 1* (*PvPdh1*) on chromosome Pv03 is associated with a major reduction in pod shattering (Parker et al. 2020). The shattering resistance allele is found at high frequency in race Durango, but is nearly absent in market classes belonging to race Mesoamerica or the Andean gene pool (Parker et al. 2020). This is a major target for improvement in these classes. Orthologs of this Pv03 gene may also regulate pod shattering in other species, such as cowpea (Lo et al. 2018), chickpea (Aguilar-Benitez 2020) and soybean (Funatsuki et al. 2014), where the orthologous locus plays a role in adaptation to arid climates by modifying the extent of twisting in pod valves (Funatsuki et al. 2014; Bandillo et al. 2017; Zhang and Singh 2020). A possible second locus on chromosome Pv08 in Middle American beans has been proposed to reduce pod shattering (Parker et al. 2020), but a relatively small sample size of these individuals has hindered the study of this allele. The Pv08 QTL is also believed to have a major effect in Andean beans, so a deeper investigation of this QTL could provide insight on whether it has evolved in parallel between domestication events.

A recently discovered QTL on Pv05, in immediate vicinity of *PvMYB26*, is associated with a loss of dehiscence in the Andean gene pool (Rau et al. 2019; Parker et al. 2020; Di Vittori et al. 2020). This locus was mapped in detail in a biparental recombinant inbred population (Rau et al. 2019), which also found significant QTLs on Pv04 and Pv09 in the same population. The role of the Pv05 and Pv09 loci were identified in parallel in a diversity panel of Andean beans (Parker et al. 2020), which also identified significant loci on Pv03 and Pv08. *PvMYB26* was subsequently found to be differentially expressed between dehiscent and non-dehiscent individuals, leading to major differences in development of cell walls in the suture (Di Vittori et al. 2020). Other loci, including *St* (controlling strings) and *To* (controlling tough pod walls), control strong fiber development in pod sutures and pod walls (Prakken 1934), respectively, and the mutant variants are found only in snap beans grown as a vegetable. *St* has been mapped to Pv02, and *To* has been mapped to Pv04 (Koinange et al. 1996; Gioia et al. 2013; Hagerty et al. 2016; Rau et al. 2019). The extreme remodeling of pods by *St* and *To* eliminates pod shattering, but makes pods extremely difficult to thresh (Emerson 1904; Prakken 1934; Murgia et al. 2017), and the alleles are therefore impractical for dry bean market classes.

Since arid conditions are predicted to increase in coming decades (Sherwood and Fu 2014), shattering-resistance alleles will be of increasing value for plant breeders. Despite this, little information exists on the degree of pod shattering in major market classes, the pattern of dominance and epistasis between resistance alleles, or the diversity available at each of these loci. Crucially, breeders also still lack genetic assays to evaluate the trait in segregating populations. Addressing these barriers will be critical to improve the productivity of a major source of nutrition globally.

Materials and methods

Plant materials and phenotyping

Three populations were evaluated in this study: a biparental population and two diversity panels, which represent each of the two domestication events of common bean. The biparental population was developed to study two shattering-related QTLs, their patterns of dominance and their interactions. Cultivars ‘Mayflower’ (ecogeographic race Mesoamerica, Kelly et al. 1989) and ‘Bill Z’ (race Durango, Wood et al. 1989) showed total resistance to pod shattering when field-grown in Davis in 2017 ($n = 27$, $n = 19$, respectively). These varieties were among the most distantly related accessions in the MDP, with neither showing any evidence of admixture

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between ecogeographic races (Moghaddam et al. 2016; Parker et al. 2020). Mayflower is a navy bean type (white, small-seeded), which possesses a SNP allele on Pv08 that is weakly associated with resistance to pod shattering in race Mesoamerica. Bill Z is a pinto bean type and has a SNP variant on Pv03 associated with strong *PvPdh1*-mediated shattering resistance common in race Durango. The population can therefore be used to determine if a reduction in pod shattering was independently selected in each of these ecogeographic races. An F_3 population of 138 individuals was developed by hybridization between these cultivars. Each F_3 individual was descended from a distinct F_2 plant, and all of the F_2s were the progeny of a single F_1 developed by cross-pollinating Mayflower and Bill Z. This 138-member Mayflower x Bill Z (MxB) population was used to validate the possible alleles on Pv03 and Pv08 and test any patterns of dominance and epistasis between the loci.

The two diversity panels were grown to evaluate the degree of pod shattering across diverse accessions of common bean. In 2016, 98 members of major market classes in the Andean Diversity Panel (ADP, Cichy et al. 2015) were field-grown in Davis, California, to evaluate each variety's susceptibility to pod shattering. In 2017, 278 varieties of the BeanCAP Middle American Diversity Panel (MDP, Moghaddam et al. 2016) were similarly field-grown in Davis to evaluate pod shattering. At maturity, a sample of pods (mean $n = 30$) was harvested from each variety.

Mature pods of all phenotyped varieties were harvested and then exposed to seven days of desiccation at 65 °C and a further seven days of re-equilibration to room temperature. The desiccation conditions for all varieties were identical, and desiccation was conducted using the same drying chamber. The proportion of pods dehiscing in this treatment was recorded, along with the market class of each variety. For evaluation of pod twists in the MxB population, all non-shattering pods were fractured by hand, and then, all pods were subjected to the desiccation treatment and re-equilibration again. The number of twists was counted for ten pods of each genotype, with "1" indicating a complete 360° rotation of the valve.

Genotyping and genetic analysis

DNA was extracted from young trifoliate leaves (approximately 1 cm in length) of the greenhouse-grown biparental MxB F_3 generation, using a modified CTAB protocol (adapted from Allen et al. 2006). DNA was quantified with a NanoDrop spectrophotometer and genotyped using the BARCBean6K_3 BeadChip (Song et al. 2015), yielding 5398 initial SNPs. SNPs that were missing or heterozygous in either parent or identical between the parents, were filtered from further analysis. The remaining SNPs were arranged into a linkage map using the ASMap R package (Taylor and Butler 2017). SNPs that did not map to one of the 11 major linkage groups were removed, leaving 1794 SNPs for QTL mapping. QTL mapping was conducted using the expectation maximization method (Lander and Botstein 1989) in R/qtl (Broman et al. 2003). Phenotypes for QTL mapping were generated by harvesting all the pods from each greenhouse-grown F_3 plant (mean $n = 27$ pods/plant), then subjecting them to seven days at 65 °C and seven further days of re-equilibration to room temperature. Pods that had fractured to the tip of the beak due to this treatment were counted as shattered, while those with no opening or only fissuring along the sutures were considered non-shattering. The percentage of pods that shattered in this treatment was used for QTL mapping. The maximum LOD score of 1000 randomized analyses of the data was used as a significance threshold. To test dominance, F_3 individuals were subset by genotype at highly significant SNPs, and comparisons were made between groups by *t*-test.

Patterns of diversity near *Pdh1*

Next, the 43 SNPs within 100 kb of *PvPdh1* in the MDP data set were analyzed to identify patterns of selection and diversity around the gene. To simplify and visualize the data, principal component analysis was performed on the SNPs using R. Sequence variation was converted to integer values and the imputePCA() function of the missMDA package was used to impute missing data (Josse and Husson 2016). The genotype data were also sorted to identify unique haplotypes within the populations. The degree of similarity between the PCA and haplotype diversity was then compared. Individuals with missing data for SNPs distinguishing the haplotypes or haplotype clusters were not shown in plots and not numbered in plots as they could not be unambiguously placed within any haplotype group.

CAPS marker development

The *PvPdh1* putative causal polymorphism (Pv03 position 49,125,490 on the accession G19833 reference genome v2.1; Schmutz et al. 2014; Parker et al. 2020) was used to develop a Cleaved Amplified Polymorphic Sequence (CAPS) marker for efficient screening of breeding populations. The sequence surrounding the SNP was extracted using Phytozome 12 (Goodstein et al. 2012). Restriction enzymes that would differentially cut the alternative alleles were identified using RestrictionMapper version 3.0 (https://www.restrictionmapper.org/). PCR primers were developed for the locus based on the sequence of accession G19833 (Andean, Schmutz et al. 2014), using the NCBI primer BLAST tool, and were then checked against the genome sequence of BAT93 (Middle American, Vlasova et al. 2016) to ensure that the sequences were identical and would successfully amplify members

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of both major gene pools. The sequence surrounding the SNP was amplified using the primers PDH1-TAQII-2F and PDH1-TAQII-2R (Table 1). PCR was conducted with Takara ExTaq (Kyoto, Japan) and included an initial elongation at 95 °C for three minutes, 44 cycles with denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, elongation at 72 °C for 60 s, and a final elongation of 72° for five minutes. PCR products were cleaved with ChimerX *Taq*II (Madison, WI, USA) during a 65 °C degree incubation for seven hours, and run on a 2.5% agarose gel.

The SNPs tightly linked to *PvPdh1* in the MDP data set were then screened for other positions that could be useful for conversion to additional CAPS markers. The SNP closest to *PvPdh1* in this data set, at Pv03 position 49,132,438 (accession G19833 genome v2.1), is distinguishable by *Eco*RI and is highly correlated with pod shattering. Unlike the *Taq*II-based CAPS marker, the allele cleaved by *Eco*RI is the shattering-resistant variant, reducing the risk of falsely identifying a susceptible individual as resistant due to technical errors in digestion. The SNP distinguished by *Eco*RI is separated from the *PvPdh1* causal polymorphism by less than 7 kb. The sequence surrounding this SNP was amplified using the primers PDH1-ECORI-1F and PDH1-ECORI-1R (Table 1). Marker development used the same methods as the *Taq*II-distinguishable marker, and the same PCR conditions successfully amplified both fragments. The amplicons were then digested by Promega *Eco*RI (Madison, WI, USA) at 37 °C for 15 min, and the PCR products were resolved on a 2.5% agarose gel.

Results

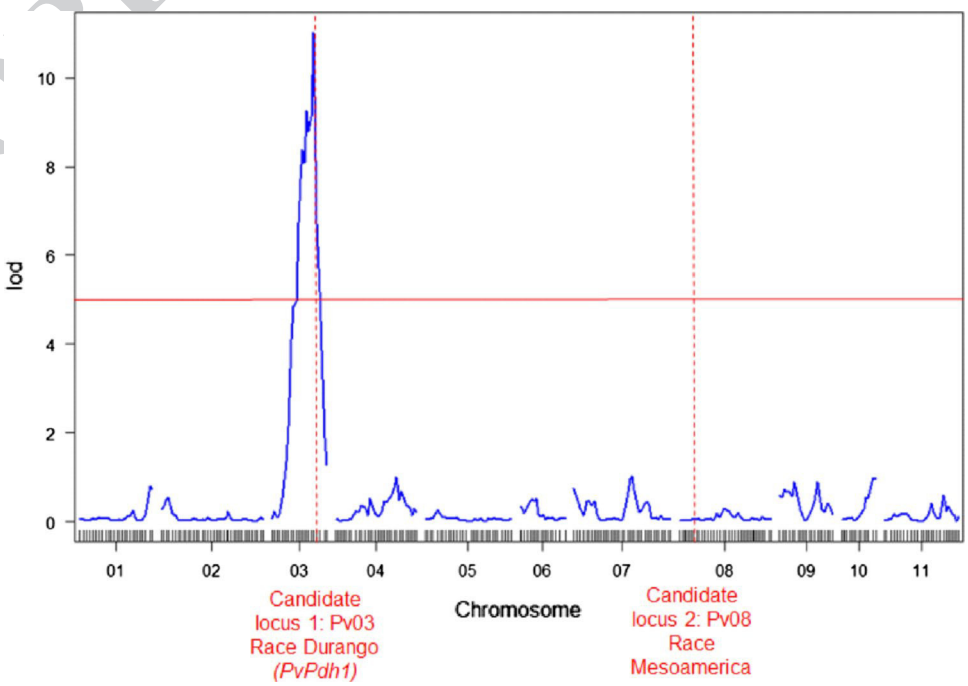
Testing multiple origins of Middle American shattering resistance and allelic effects

Only one major QTL was associated with pod shattering in the MxB population, indicating that strong resistance to pod shattering may have arisen only once in Middle American domesticated common beans. The most significant SNP was found on chromosome Pv03 (Fig. 2, ss715646441; Pv03 48,944,785 bp, Supplementary Table S1, Song et al. 2015), 181 kb from the putative *PvPdh1* mutation (Pv03 49,125,490 bp, Parker et al. 2020). This was the closest segregating SNP in physical distance to *PvPdh1*; it had a LOD score of 10.4. On the other side of *PvPdh1*, the next

Table 1 Primers used for CAPS marker analysis

Primer	Sequence	Amplicon	Primer length	Primer T_m
PDH1-TAQII-2F	TTCGACCTTCCCACTCCAGA	PDH1-TAQII	20 bp	60
PDH1-TAQII-2R	AGACGAGGCTGTTGACAGAA	PDH1-TAQII	20 bp	59
PDH1-ECORI-1F	AAGTTGGAAGTGGCTGCTGT	PDH1-ECORI	20 bp	60
PDH1-ECORI-1R	GGGAAAGCCACAAAGGCATC	PDH1-ECORI	20 bp	60

Fig. 2 .



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segregating SNP (ss715647338, 50,390,256) was both considerably further from the candidate gene (1.26 Mb) and had a much lower LOD score (LOD = 7.1). This includes a relatively wide interval of 145 genes. No QTL was identified on Pv08 (Fig. 2). The highest LOD score on this chromosome was 0.231, far lower than the significance threshold of 5.09.

The number of pod twists was positively correlated with proportion shattering in the desiccator (simple linear regression, $p = 0.0012$, Fig. 3a). Allele at ss715646441 explained 40.4% of the variation in pods shattering in the desiccator, and 7.2% of variation in pod twists. No significant difference in proportion of pods shattering existed between heterozygotes and homozygous-susceptible types at ss715646441 (*t*-test, $p = 0.14$). In contrast, heterozygotes had significantly higher rates of pod shattering than types homozygous for the shattering-resistant allele (*t* test, $p = 9.0*10^{-12}$, Fig. 3b,c). This indicated that *PvPdh1*-mediated pod shattering resistance is recessive. In contrast, the number of pod twists showed the opposite dominance pattern, with no significant difference between heterozygotes and low-twisting homozygotes (*t* test, $p = 0.83$, Fig. 3d, e). Heterozygotes were significantly different than high-twisting homozygotes (*t* test, $p = 0.01$, Fig. 3d, e). This indicates that the dominance of alleles varies based on phenotyping method.

Pod shattering by market classes

Major discrepancies in pod shattering exist between the major market classes of common bean (Fig. 4, Table 2). In the Andean gene pool, pod shattering is highest in the cranberry market class, with a mean value of 41% of pods shattering after desiccation. The purple speck/mottled market class has the greatest degree of shattering resistance among Andean beans, with only 3% of pods shattering after the same treatment. In Middle American beans, pod shattering is highest in the black (18% shattering) and navy/small white (15%) market classes of race Mesoamerica, and lowest in the pinto (1%), great northern (1%) and pink (2%) classes of race Durango. *PvPdh1*-mediated resistance to pod shattering is found almost exclusively in pinto, great northern, and pink market classes (Parker et al. 2020) and is therefore associated with levels of pod shattering which may be the lowest of those of any major economic groups of common beans grown for grain.

Diversity around *Pdh1*

Three major SNP haplotype clusters were identified in the sequence surrounding the *PvPdh1* gene (Fig. 5a). The most distinct of these included six individuals, several of which are of known Andean ancestry. The first principal component of the genetic data explained 64% of the variation and separated this group from the two other major clusters. The second principal component explained 25% of the variation and separated varieties belonging to race Mesoamerica from race Durango. Five individuals with missing data for the ten SNPs that distinguish these ecogeographic races were filtered from subsequent analyses. Additionally, cv. 'Tepary 22′ (*Phaseolus acutifolius*) and cv. 'Jackpot' (*Phaseolus vulgaris*) exhibited highly unique haplotype patterns. Jackpot shows recombination in the region between the predominant haplotype of the Andean gene pool and race Durango, while *P. acutifolius* is a separate but closely related species (Freytag and Debouck 2002; Delgado-Salinas et al. 2006).

Races Durango and Mesoamerica differed in haplotype diversity around the *PvPdh1* locus (Fig. 5b, c). The race Mesoamerica haplotype cluster includes six unique haplotypes. The most common of these includes 137 of the 148 varieties that can be clustered into a group unambiguously (93%), without missing data in the SNP positions distinguishing the sub-groups. The race Mesoamerica haplotypes displayed 18% shattering on average. In contrast, race Durango varieties display only three haplotypes. The most common of these haplotypes includes 178 of 182 unambiguous varieties (98%), with an average proportion shattering of 0.6% in this group. The two low-frequency race Durango haplotypes showed no shattering when field-grown in 2017 (three varieties phenotyped, 0% pod shattering in each, one variety with no data).

CAPS marker development

The *Taq*II-based CAPS marker of the *PvPdh1* causal polymorphism leads to cleavage of susceptible alleles, while resistant alleles are not cut. The total pre-digestion amplicon length in G19833 was 578 bp, comparable to the 580 bp amplicon of BAT93. After digestion, susceptible alleles were cleaved into fragments of 449 and 129 bp in G19833. While digestion was seen in all shattering-susceptible samples after digestion with *Taq*II, this enzyme led to only partial digestion in a minority of cases. The *EcoRI*-digestible CAPS marker was extremely robust, and never led to partial digestion. After digestion, this marker led to resistant alleles that were cut into fragments of approximately 332 bp and 310 bp, while susceptible alleles remained uncleaved at 642 bp in BAT 93 (Fig. 6). Andean varieties showed comparable fragment sizes, such as 639 bp in G19833. The *EcoRI*-digestible CAPS marker never experienced partial digestion or ambiguity. The SNP used for this marker has a strongly significant correlation with pod shattering (*t* test, $p = 4.7*10^{-33}$), and is one of the 10 SNPs contributing to the haplogroup differentiation between race Durango and race Mesoamerica (Fig. 5b, c). The median proportion of pod shattering among 97 varieties with the shattering susceptible allele was 0.14, equal to the maximum level of shattering seen in any of the 160 varieties carrying the resistant allele (Fig. 7). The AQ7

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Fig. 3 .

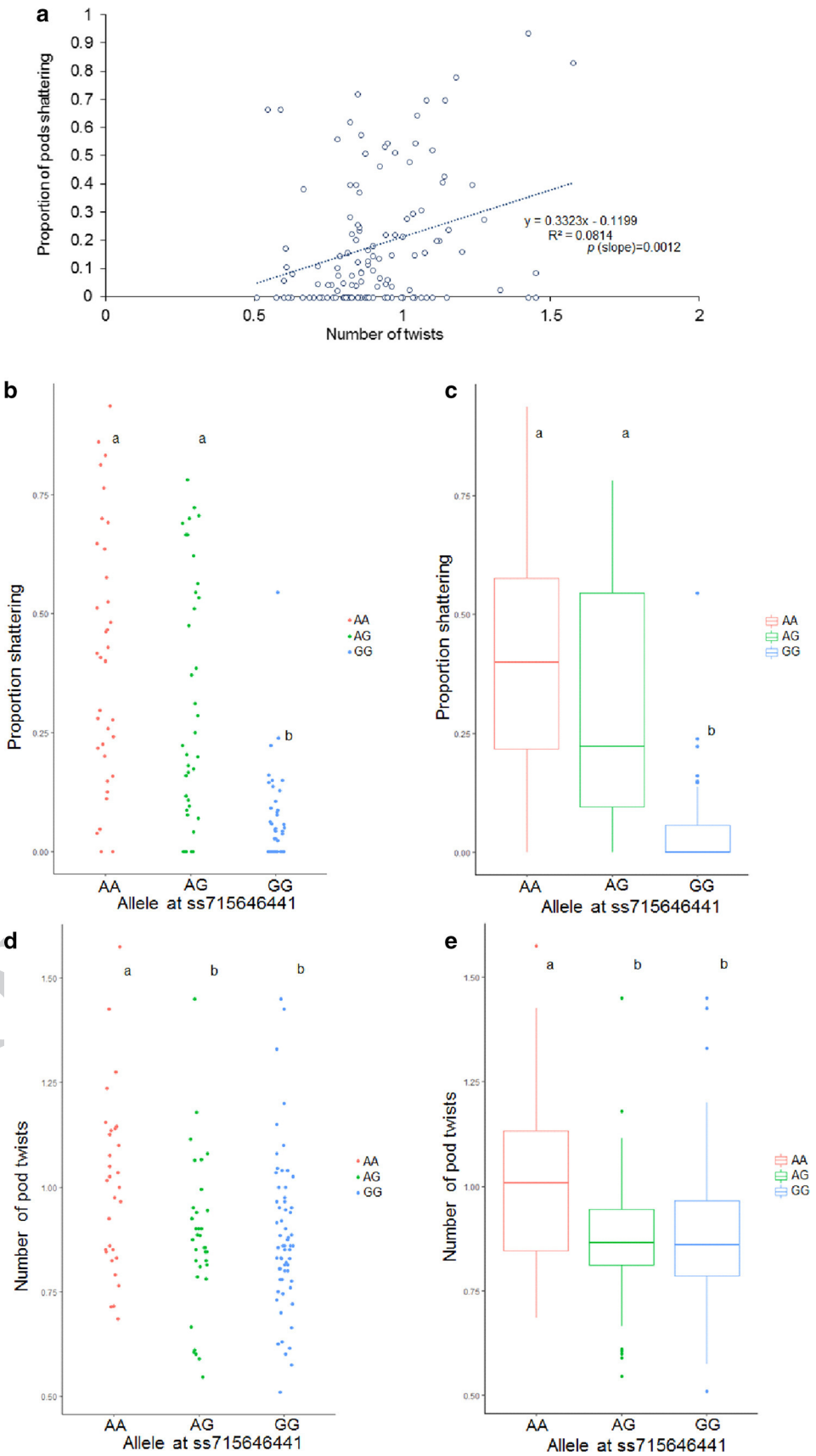

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Fig. 4 .

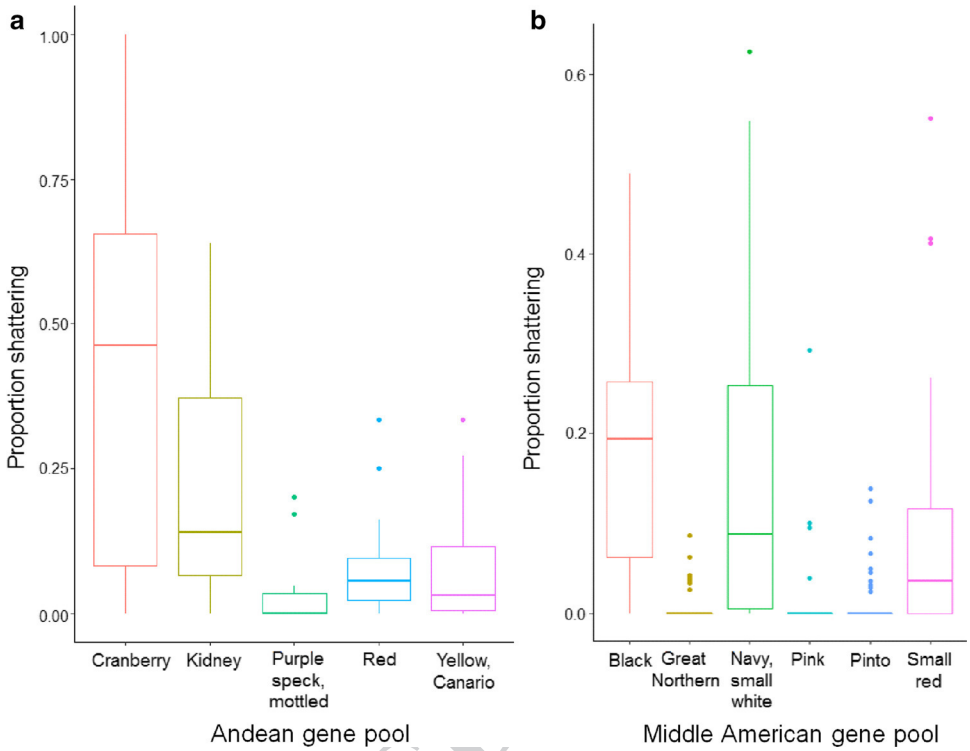

Table 2 Pod shattering (PS) after desiccation, by market class, gene pool and ecogeographic race

Market class	*N* (accessions)	Gene pool	Race	Mean PS (%)	Median PS (%)	St. dev. (%)
Cranberry	24	Andean	Nueva Granada	41.43	46.29	29.86
Kidney	43	Andean	Nueva Granada	21.09	13.89	18.32
Purple speck/mottled	17	Andean	Nueva Granada	3.11	0	5.84
Red	22	Andean	Variable	7.54	5.51	8.1
Yellow/canario	14	Andean	Variable	8.45	3.04	10.54
Great northern	31	Middle American	Durango	0.94	0	2.12
Pink	23	Middle American	Durango	2.48	0	6.37
Pinto	93	Middle American	Durango	0.74	0	2.38
Black	43	Middle American	Mesoamerica	17.63	19	13.22
Navy/small white	46	Middle American	Mesoamerica	15.2	8.5	16.62
Red/small red	29	Middle American	Variable	9.59	4	14.7

median proportion shattering of those varieties with the resistant allele was 0.00%.

Discussion

Testing for multiple origins of Middle American shattering resistance and allelic effectsOnly one major locus was identified with an effect on pod shattering in the Middle American domesticated gene pool of common bean. These results highlight the important role of the Pv03 *PvPdh1* locus in this population. The lack of a major QTL on Pv08 in the MxB population suggests that Mayflower has no shattering resistance allele on that chromosome which is not also found in the distantly related Bill Z. Because the Pv08 SNP identified through GWAS did not reach significance after a Bonferroni correction (Parker et al. 2020) and only 11 of 280 members of the MDP possessed this SNP, our results indicate that this chromosome does not have a major, widespread role in regulating pod shattering in the Middle American domesticated gene pool. This does not preclude the possibility that the QTL has a role in shattering of Andean beans, the latter of which has been demonstrated with much greater confidence (Parker et al. 2020). The locus may also have a role in regulating pod shattering in a very small proportion of Middle American bean varieties,

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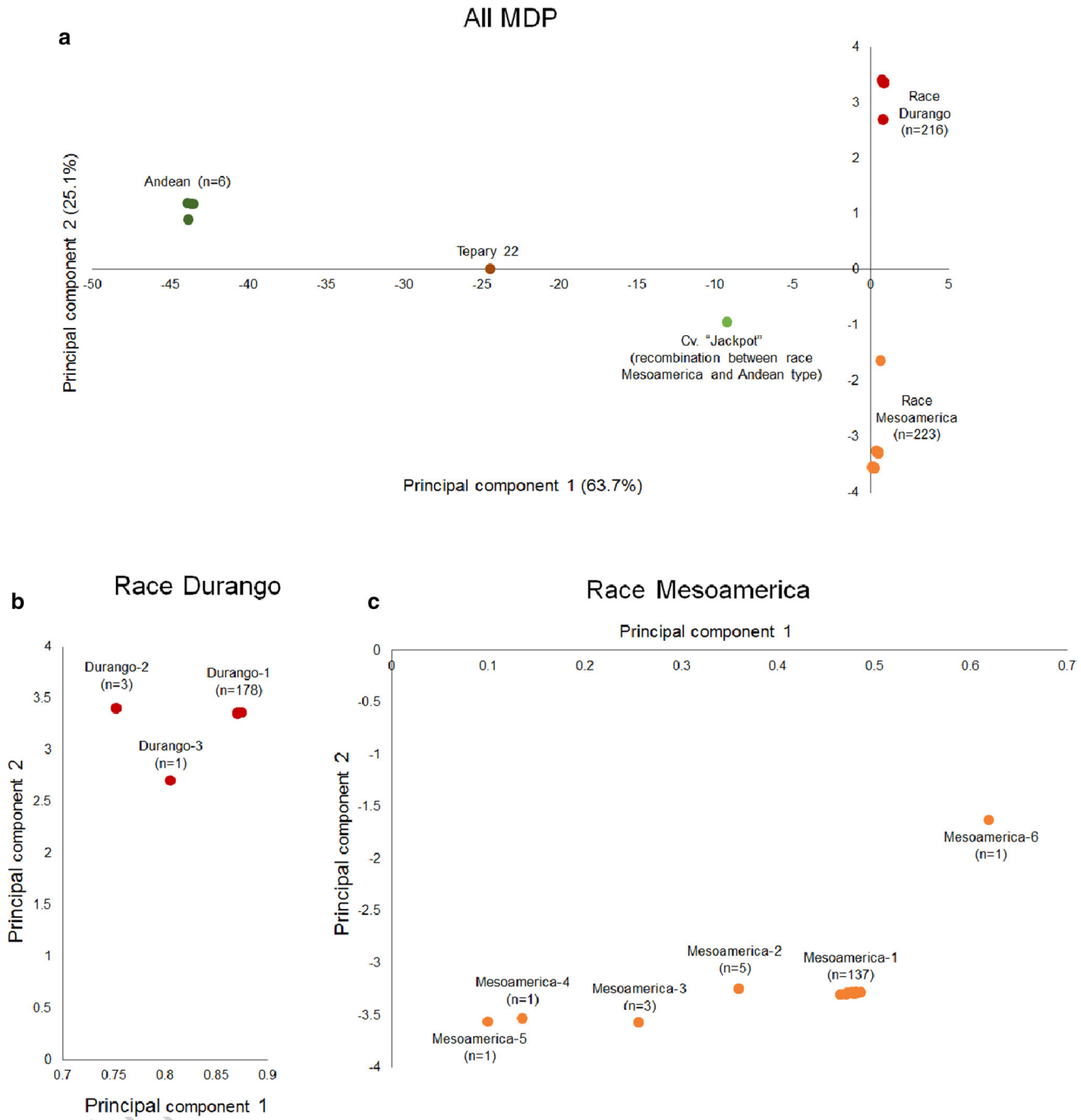

Fig. 5 .

possibly through de novo mutation in race Mesoamerica or introgression from the Andean gene pool. The role of Pv08 in the original domestication of Middle American common beans also cannot yet be ruled out. Our Pv03 QTL mapping peak in the MxB population was closer in physical distance to *PvPdh1* than what has been previously identified through QTL mapping (Parker et al. 2020) in a different recombinant inbred population—ICA Bunsi x SXB405 (Berny Mier y Teran et al. 2019). These results are still not as close to the gene as those achieved by GWAS with a much larger SNP dataset (Parker et al. 2020).

The correlation between *PvPdh1* allele and pod twists indicates that the gene may modify the twisting force of pod walls. This has been seen in the soybean ortholog (Funatsuki et al. 2014) as well as across numerous legume species (e.g., Murgia et al. 2017; Rau et al. 2019; Takahashi et al.

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Fig. 6 .

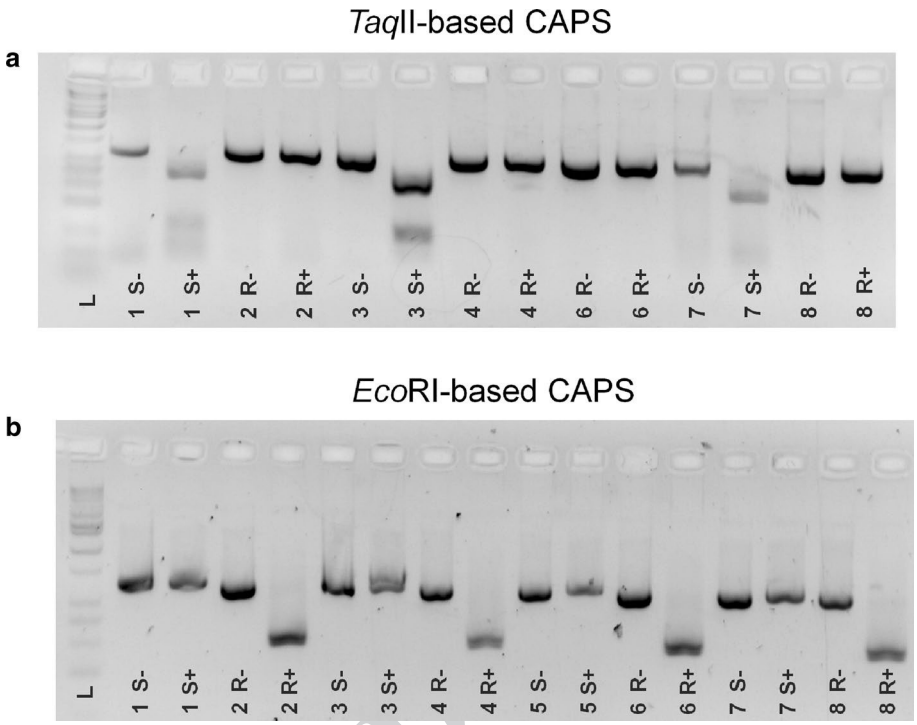
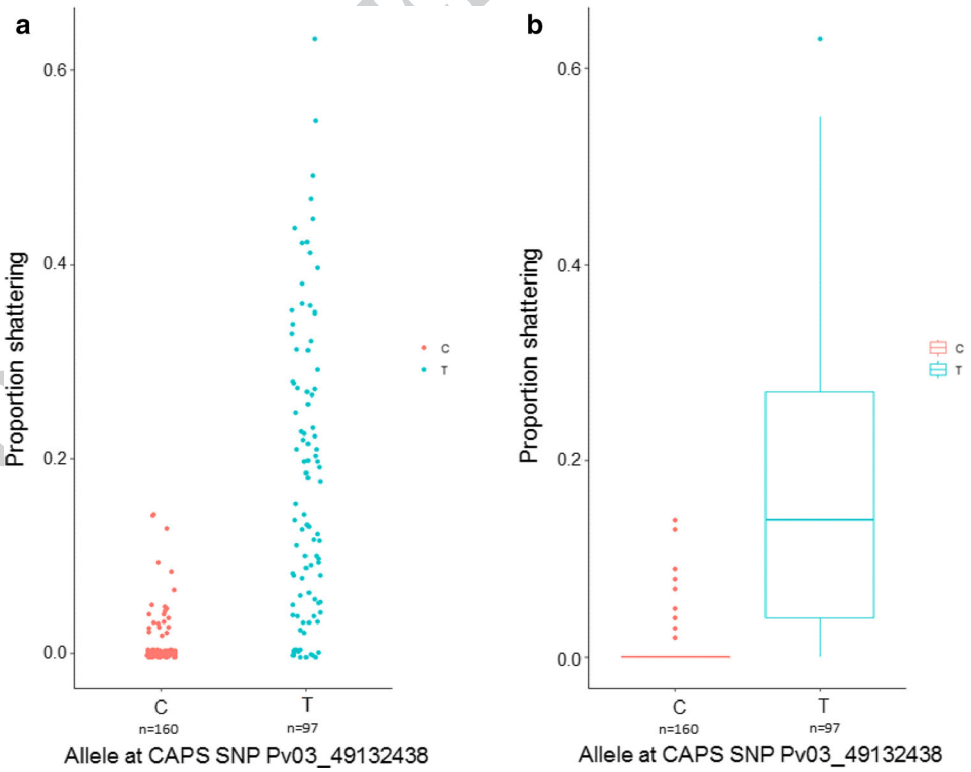


Fig. 7 .



2019a, b). The complex dominance of *PvPdh1*-mediated shattering resistance parallels the pattern seen in soybean pods, in which the phenotyping method affects the pattern of dominance (Funatsuki et al. 2014). The desiccation method was faster to phenotype than counting pod twists and also produced results which were much more correlated with genotype. This indicates that the desiccator method may be a more effective method of phenotypic screening than counting twists. The recessive nature of pod shattering when phenotyped by the desiccator method means that carriers of the

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resistant *Pvpdh1* allele may demonstrate high levels of pod shattering in early breeding program generations because of heterozygosity and should not be eliminated without direct genetic evaluation or subsequent progeny tests. Further, the recessive nature of shattering resistance when phenotyped by the desiccator method also indicates that recurrent backcrossing based on phenotyping alone would not be practical for the trait. *Pvpdh1* therefore requires a genetic marker for screening of progenies that carry the shattering-resistance allele.

Pod shattering by market class

Our results indicate that durable resistance to pod shattering has evolved independently in both the Middle American and Andean gene pools of common bean. Despite this, many varieties in both gene pools continue to display the wild-type propensity to shattering, and this is strongly associated with market class. Our results agree with earlier anecdotal observations that pod shattering is most problematic in the black and cranberry market classes (Temple and Gepts 2012), the two categories with the highest rates of pod shattering in the MDP and ADP. In contrast, market classes with the lowest rates of pod shattering are those in which the resistant *Pvpdh1* allele is most abundant (Parker et al. 2020). While direct comparisons between the Andean and Middle American gene pools are complicated by the fact that the populations were grown in different years, the desiccation treatment used to induce pod fracture was identical between populations. In any case, it is clear that many varieties of both gene pools experience high levels of pod shattering and would benefit from the introgression of shattering-resistance alleles. Market demands require most new varieties of common bean to conform to standards for several complex traits, such as seed size, shape, color, leading most modern breeding to focus preferentially on intra-race crosses. Marker-assisted backcrossing would greatly facilitate the transfer of the shattering-resistant allele into other ecogeographic races, while maintaining the complex genetic background required in a market class. A better understanding of the *PvPdh1* locus, as well as molecular markers associated with it, will become increasingly important for crop improvement as conditions become more arid in the twenty-first century.

Haplotype diversity

Our haplotype diversity results are consistent with the hypothesis that there has been stronger selection pressure on *PvPdh1* in race Durango than in race Mesoamerica. After selection of the shattering resistant allele at *PvPdh1,* race Durango types differentiated into just two additional new haplotypes, which represent 3% of the group's sampled varieties. The non-shattering character found in the low-frequency haplotypes indicates that these groups may have differentiated since the mutation in *PvPdh1*, rather than being ancestral relicts of a shattering-susceptible race Durango progenitor. In contrast, race Mesoamerica includes six total haplotypes, and the five least common of these together represent 7% of the sampled varieties. This is more than double the frequency of minor haplotypes than in race Durango. These less-common variants could be the subject of future study to identify whether secondary mutations in *PvPdh1* have independently arisen to regulate pod shattering in a subset of varieties within race Mesoamerica.

CAPS marker development

While *Eco*RI is a highly stable, robust enzyme, *Taq*II is a high molecular weight, lower-stability enzyme, which requires highly specific conditions for optimal DNA cleavage (Roboklon 2020, ChimerX 2020). This includes a pre-digestion PCR product cleanup and extreme care in handling of the enzyme. Although *Taq*II treatment always led to digestion of susceptible alleles, this digestion was sometimes only partial, leading to ambiguity between homozygous susceptible and heterozygous individuals. Further, the cleavage of shattering-susceptible alleles (such as by *Taq*II) is generally less desirable than cleavage of resistant alleles (such as by *Eco*RI) to reduce the risk of selecting susceptible types due to technical errors. While the *Taq*II-based marker may be ideal for initial parental screening, the tightly linked *Eco*RI-based CAPS marker may be more practical for rapid, efficient screening of large breeding populations.

The CAPS markers developed here may be valuable for rapidly transferring the pod-shattering resistance of race Durango into the market classes of race Mesoamerica and the Andean gene pool. Pod shattering is a complex quantitative trait and is regulated by multiple alleles and environmental variables. Indeed, selection based on phenotyping alone will not always be predictive of an individual's susceptibility to pod shattering (Figs. 3, 6), leading to imperfect selection accuracy. Our CAPS markers will provide a more accurate and rapid method to genetically evaluate an individual's resistance to pod shattering. Similarly, the SNPs used to develop these CAPS markers could be converted to Kompetitive Allele Specific PCR (KASP) markers through commercially available services. Phenotypically, this trait cannot be measured until after plants have fully senesced, delaying selection and requiring breeders to invest heavily in non-desired plants. Further, it often requires additional heat treatment incubation periods or labor-intensive analyses with specialized equipment, such as mechanical force measurement gauges, to study accurately (e.g., Funatsuki et al. 2014; Dong et al. 2014; Parker et al. 2020). In contrast, our genetic tests can be conducted rapidly on segregating populations of seedlings, reducing costs for breeding programs

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and hastening genetic improvement. These markers will also allow breeders to accurately pyramid shattering resistance alleles from the Andean and Middle American gene pools for the first time, potentially leading to stronger resistance to pod shattering than what is provided by *Pvpdh1* alone. In turn, this will facilitate the development of varieties that are more tolerant of warm, dry environmental conditions where pod shattering is most problematic.

Acknowledgements Seeds of the ADP and MDP were provided by R. Lee and P. McClean (North Dakota State University). Jorge Carlos Berny Mier y Teran provided advice during linkage map construction. Funding for T.A.P. was provided through a Clif Bar Family Foundation Seed Matters fellowship and Lundberg Family Farms research support. Additional funding was provided by Henry Jastro research awards through the UC Davis Plant Biology Graduate Group. Funding for Lorenna Lopes de Sousa was provided through CAPES, Brasília, Brazil.

Author contribution statement T.A.P. prepared the manuscript and conducted or co-conducted all elements of the research. L.L.d.S helped with greenhouse management, DNA extraction, and pod phenotyping. T.d.O.F. contributed to pod phenotyping and CAPS marker development. A.P. contributed to field and greenhouse management. P.G. guided the project. All authors edited the manuscript.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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