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Pod physical traits significantly implicate shattering response of pods in beans (*Phaseolus vulgaris* L.)

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Abstract

Pod shattering is an undesirable process leading to loss of harvestable yields. In the present study, we sought to undertake the first comprehensive phenotyping in 254 bean (*Phaseolus vulgaris* L.) genotypes for pod shattering including various mechanistic aspects as well as assess natural variation in the germplasm set for 16 seed physical traits including shattering score. There was substantial variability in 16 pod physical traits in the material. Significant diversity of the material in respect of pod traits was indicated by the broad range and coefficient of variation (CV) values. Using Random Impact Assessment (RIA), we found substantial variability in pod shattering score in common bean genotypes indicating significant diversity. Shattering score had a mean value of 6.098 with a range of 1.07 to 9.13. Highest shattering score was recorded in WB-6, WB-20-247, and N-7 while the lowest value for shattering score was recorded in WB-1129 and WB-216. Shattering score was negatively correlated with pod thickness (r = -0.698) followed by ventral/dorsal length ratio (r = -0.468) and positively correlated with breadth/thickness ratio (r = 0.599) and string % (r = 0.590). The principal component analysis (PCA) concentrated 86.91% variability in the first five principal components, and the first two PCs accounted for 55.62% of the total variation.

Keywords: common bean, domestication syndrome, pod physical traits, pod shattering, seed dispersal.

Introduction

Common bean (*Phaseolus vulgaris* L.) is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit (both commonly called beans). The main categories of common beans, on the basis of usage, are dry beans (seeds harvested at the completion of maturity), snap beans (tender pods with reduced fiber harvested before the seed development phase), and shelled beans (seeds harvested at physiological maturity). The common bean is a highly variable species that has a long history of cultivation. All wild members of the species have a climbing habit, but many cultivars are classified either as bush beans or dwarf beans, or as pole beans or climbing beans, depending on their style of growth. These include the kidney bean, the navy bean, the pinto bean, and the wax bean. The other major types of commercially grown beans are the runner bean (*Phaseolus coccineus*) and the broad bean (*Vicia faba*). Most varieties grow either as an erect bush or as a climbing plant. When the climbing type is grown for its immature pods, such as for green beans, artificial supports are necessary to facilitate harvesting. Global production of about 24 million tons mainly comes from Sub-Saharan Africa and Latin America, accounting for about half of the common bean production followed by South and South-East Asia (35%). Global exports of

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Abbreviations: BTR - breadth thickness ratio; CV - coefficient of variation; DPL - dorsal pod length; FPM - filled pod mass; LBR - length breadth ratio, PB - pod breadth; PL - pod length; PT - pod thickness; PW% - pod wall; PWM - pod wall mass; PWT - pod wall thickness; RIA - Random Impact Assessment; SHS - shattering score; SM - seed mass; SP - string %; SPR - seed pod ratio; VDR - ventral/dorsal ratio; VPL - ventral pod length.
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common bean stand at 4.23 million tons (31%), only next to pea among pulses (Rawal and Navarro 2019).

Pods are important in terms of reproductive fitness of legumes especially beans. They not only perform a protective function by encapsulating the developing seeds and protecting them from diseases and pest but, the pod walls, being photosynthetically active, contribute assimilates and nutrients to growing seeds. In fact, recent evidences point towards relay of signals from the pod stimulating coordinated grain filling as well as regulation of reallocation of reserves from damaged seeds to those that have retained viability. Thus, pods can regulate seed growth and maturation, and explore how the timing and duration of pod development might be manipulated to enhance either the quantity of crop yield or its nutritional properties (Bennett *et al*. 2011).

During the process of domestication of crops from wild, human driven accelerated selection for desired plant traits has led to the loss of several adaptive traits, such as seed shattering, in plants that are otherwise vital for persistence under wild conditions (Gregory 2009, Flint-Garcia 2013). Shattering as a process is the dispersal of a crop's seeds upon their becoming ripe. In wild systems, it is desirable from the point of dispersal of seed as well as survival in new places. However, under domesticated system, it is generally an undesirable process as it leads to loss of harvestable yields. Shattering resistance has evolved under domestication as a result of series of mutations that reduced the dispersal of seeds as soon as they were ripe, with the mutants retaining the seeds for longer, making harvesting much more effective. Even in modern breeding programs focusing on transfer of traits from wild to broaden the genetic base, ensuring non-shattering phenotype is one of the imperatives especially when introgressing valuable traits from wild varieties to domesticated crops.

Among various domestication syndromes, pod shattering is the most important one. The evolution of shattering resistance has occurred independently in several crops and in different areas of the world during the process of domestication of food crops, as this loss has proved to be the important driver of the adaptation of the plants to the agro-ecosystem, to provide ancient farmers with easier and more abundant harvests (Tang *et al*. 2013). Gioia *et al*. (2013) concluded that evolution of resistance to pod shattering represents a key component of the domestication syndrome in common bean (*Phaseolus vulgaris* L.) and makes this domestication dependent upon the farmer for seed dispersal. Indehiscent phenotype emerged in common bean, which was domesticated in the new world (Hymowitz 1970). However, fully indehiscent phenotype emerged in common bean only after domestication with the development of snap varieties that are used for the production of green beans due to the absence of fiber strings along the pod valves. As a domestication trait, shattering resistance confers advantage in terms of ease of harvest, survival in varying environments, and increased yield (Ogutcen *et al.* 2018).

In legumes, shattering of pods is triggered by the hygroscopic movements within the pod valves following dehydration. The release of the accumulated elastic tension during dehydration results in the splitting of the valves along their suture lines (Elbaum and Abraham 2014). Evolutionarily, pod in common bean has evolved from a single leaf, where the leaf folds to cover the seeds (Christiansen *et al.* 2002, Sofi *et al.* 2022). The two halves are connected by ventral and dorsal sutures of the bean pod. Among the two sutures, ventral is very important in respect of pod shattering. It is a modified midrib, while the dorsal suture corresponds to the fused margins of a modified leaf (Carlson and Lersten 2004). The vascular bundles develop thick walls at the sutures and the resulting structure is called the bundle. Anatomically, there are visible fissures on ventral side that cause separation of pod valves upon pod maturity (Sofi *et al.* 2022).

A number of researchers have provided insights about plant and pod traits that influence pod shattering response in different crops such as loss of adhesion between cells (Agrawal *et al*. 2002), cell separation (Swain *et al*. 2011), length, width and volume/mass ratio (Bara *et al*. 2013), lignification of fibre cap cells (Dong *et al*. 2014), pod succulence and fibre content (Singh and Singh 2015), pod wall mass and pod wall water content (Kuai *et al*. 2016), pod number, thickness of the pod, and seed mass/pod mass ratio (Krisnawati et al. 2019), pod thickness (Zhang *et al.* 2018), pod length (Krisnawati *et al.* 2020). However, in common bean, not much work has been reported on pod physical traits *vis-a-vis* pod shattering response, even though substantial work has been done in soybean.

In the present study we sought to undertake first comprehensive phenotyping in Western Himalayan common bean collection for pod shattering including the various mechanistic aspects as well as assess natural variation in the available germplasm set. The study was undertaken as the pod shattering is the most significant domestication syndrome trait in common bean and apart from snap beans, dry beans have retained substantial shattering and may cause severe losses (up to 100%) in legumes including common bean. More importantly, increasing temperatures under climate change scenario will exacerbate the problem of pod shattering (Lo *et al.* 2021) especially under arid environmental conditions. The broad hypothetical framework of present study was that the diverse bean germplasm has domesticated under diverse ecological conditions and has diverse shattering response and as a trait, pod shattering has definite physical, anatomical, and biochemical parameters that can be used as effective surrogates for improving pod shattering.

Materials and methods

Site of the experiment: The experiment was laid in 2021 at the research fields of Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, SKUAST-K, Sopore (34°17' N and 74°33' E at an altitude of 1 594 masl). The soil of the experimental site is a typical inceptisol with clay loam texture. The pH was almost neutral (7.2), with organic carbon 0.65%, electrical conductivity of 0.18 dS/m and CEC of 16 meq/100 g. All the accessions were grown as single rows of four-meter length, with a spacing of 15 × 40 cm, in an augmented block design with four checks. The mean minimum and maximum temperatures (May - September) were 10.63 and 22.48°C, with the lowest (16.43°C) and highest (25.61°C) maximum recorded in May and July.

Plants: The material for the present study comprised of a core set of 254 lines including four checks (two state-released checks Shalimar Rajmash-1, Shalimar French Bean-1 and two nationally released varieties Arka Anoop and Arka Komal), representing diverse market classes in beans. The accessions belonged to both plain seeded as well as mottled beans ranging across diverse color classes and seed sizes and shapes. All the accessions were cultivated species belonging to *P. vulgaris* and comprised both released varieties, breeding lines, traditional landraces, and gene bank accessions. All the accessions were grown as single replicates in an augmented block design except the checks that were replicated in each block.

Crop management: The management practices were uniform and homogeneous and comprised of seed treatment with the fungicide and the insecticide at the rate of 2 ml/kg(seed), application of the pre-emergent herbicide *Pendimethalin* at a dose of 1.25 L/ha as well as timely manual weeding, recommended dose of fertilizers (NPK) comprising a basal dose and a topdressing of urea at the V3 stage (first open trifoliate leaf). The crop was irrigated intermittently to avoid drought stress that would have confounded the results. The pods were harvested manually at the R9 (maturation stage), when 95% of pods were physiologically mature. Ten pods were put in paper bags (20 × 10 cm) where they equilibrated to constant moisture content for 10 days at room temperature.

Manual screening for pod shattering using random impact method (RIM): A major reason of lack of progress in development of shattering resistant beans was obvious lacuna in breeder's assessment of susceptibility to shattering that relies mainly upon visual observations in the field or upon hand testing of pods (Child *et al.* 2003). Field based phenotypic evaluation of pod shattering requires fully grown plants, and it is a time-consuming and labor-intensive procedure (Kim *et al*. 2020). Moreover, the fluctuations in weather parameters at the time of pod maturation causes bias in the results. However, a test procedure, namely Random Impact Assessment (RIA) has been devised that exposes pods to random impacts in a similar manner to those that occur in the crop canopy during harvest (Bruce *et al.* 2002, Murgia *et al.* 2017). This RIM enables the rapid comparison of susceptibility to shatter in samples of fully mature pods from individual plants. In the present study, screening of pod shattering was done in laboratory using RIA method suggested by Murgia *et al.* (2017) with modifications using an in-house designed RIA comprising of a 20 cm diameter cylinder with six steel balls of 12-mm diameter. The method exposes pods to random impacts in a manner similar to those that occur in the crop canopy during harvest. This RIM enables the rapid comparison of shattering response of fully mature pods from individual plants (Bruce *et al.* 2002). The pods harvested at maturity and equilibrated for moisture were oven dried at 80°C for 2 d and 10 sampled pods were put in RIA and manually shaken for 10 s using a stopwatch. Each treatment was done in triplicate. The percentage of pods shattered was recorded as an estimate of pod shattering resistance. Data were recorded both before and after shaking the apparatus.

Results

Variability for pod physical traits: There was substantial variability in 16 pod physical traits in 254 common bean genotypes indicating significant diversity of the material in respect of pod traits (Table 1). Pod length (PL) had a mean

Pod physical traits recorded in the present study.

Traits	Units	Measured as
Pod length	cm	average length of pods was calculated in cm using measuring scale
Pod breadth	cm	average breadth of pods was calculated in cm using vernier caliper
Pod thickness	cm	average thickness of pods in cm using vernier caliper
Length/breadth ratio	-	ratio of length of the pod to its respective breadth
Breadth/thickness ratio	-	ratio of breadth of the pod to its respective thickness
Ventral pod length	cm	average ventral length of pods was calculated in cm using measuring scale
Dorsal pod length	cm	average dorsal length of pods was calculated in cm using measuring scale
Pod curvature (V/D ratio)	-	ratio of ventral side of pod to its dorsal side
String/pod proportion	%	percentage of string/pod by weighing them separately
Pod wall mass	g	average mass of pods of all genotypes using weighing balance in grams
Pod wall thickness	mm	thickness of the pod wall expressed in mm using vernier caliper
Seed pod ratio	-	ratio of pod with seed and without seed
String mass	g	average mass of string in grams using weighing balance
Pod wall percentage	%	percentage of pod wall in the genotype
Filled pod mass	g	pod mass with seed in grams using weighing balance

value of 13.381 (7.50 - 20.00 cm). The highest PL was recorded in WB-20-312 (20.12 cm), followed by KDR-98 (17.12 cm), and G-3 (17.02 cm). Pod breadth (PB) had a mean value of 7.87 cm (0.50 - 1.03 cm). The highest PB was recorded in WB-1310 (1.04 cm), followed by WB-970 (1.03 cm), and GL-3 (1.02 cm). Pod thickness (PT) had a mean value of 0.58 cm with a range of 0.48 - 0.81 cm. The highest PT was recorded in WB-435 (0.85 cm), followed by WB-1680 (0.83 cm), and WB-1239 (0.82 cm). Pod length/breadth ratio (LBR) had a mean value of 15.20 with a range of 9.76 to 28.17. The highest pod LBR was recorded in N-1 (25.04), followed by WB-20-312 (22.52), and KD-13 (22.51). Pod breadth/thickness (BTR) ratio had a mean value of 1.54 with a range of 0.92 to 2.04. The highest pod BTR was recorded in WB-22 (1.71), followed by WB-20-176 (1.59), and WB-508 (1.56).

Dorsal pod length (DPL) had a mean value of 13.32 cm with a range of 7.30 to 20.00 cm. The highest DPL was recorded in WB-20-312 (20.00 cm), followed by WB-1678 (16.94 cm), and the lowest value for DPL was recorded in WB-20-232 (7.30 cm). Ventral pod length (VPL) had a mean value of 12.58 cm with a range of 6.50 to 18.87 cm. The highest VPL was recorded in WB-20-312 (18.87 cm), followed by WB-45 (16.27 cm), and WB-119 (16.02 cm), while the lowest value for VPL was recorded in WB-20-232 (6.5 cm). Ventral/dorsal ratio (VDR) had a mean value of 0.94 with a range of 0.89 to 1.01. The highest VDR was recorded in WB-208 (1.01), followed by WB-966 (1.00), and WB-352 (0.99) and the lowest value for pod VDR was recorded in WB-20-238 (0.89). Filled pod mass (FPM) had a mean value of 13.32 g with a range of 6.47 to 19.89 g

Seed mass (SM) had a mean value of 5.33 g with a range of 1.00 to 9.40 g and the highest seed mass was recorded in WB-1439 (9.40 g), followed by WB-20-206 (8.90 g), and WB-1441 (8.87 g), while the lowest seed mass was recorded in WB-1319 (1.00 g). Pod wall mass (PWM) had a mean value of 7.98 g (0.09 to 14.78 g). The highest PWM was recorded in WB-970 (14.78 g), followed by WB-20-312 (14.69 g), and WB-20-177 (13.52 g), while the lowest PWM was recorded in WB-20-255 (0.094 g). Pod wall % (PW%) had a mean value of 1.22% with a range of 0.01 to 4.31%. The highest PW% was recorded in WB-1319 (4.31), followed by WB-970 (3.89), and WB-180 (3.78). Seed pod ratio (SPR) had a mean value of 0.73 (0.13 to 0.97). The highest SPR was recorded in WB-20-170 (0.97), followed by WB-20-206 (0.96), and WB-1644 (0.94) while the lowest SPR was recorded in SB-183 (0.13).

Genetic variability for shattering response: In the field we screened the genotypes for the level (shattering *vs.* non-shattering) and mode (twisting *vs.* non-twisting) using polyvinylchloride cages. However, in case of all the genotypes, all classes indehiscent, fissured, opened, and twisted were found. However, there was huge variation between sampled plants within each genotype. In order to create a uniform screening system, we used RIA. There was substantial variability in pod shattering score in 254 genotypes of common bean indicating significant diversity of the material in respect of studied traits (Table 1, Figs. 1, 2). Shattering score (SHS) had a mean value of 6.09 with a range of 1.07 to 9.13. The highest SHS was recorded in WB-6 (9.13), followed by WB-20-247 (8.44), and N-7 (7.66), while the lowest SHS was recorded in WB-1129 (1.07), followed by WB-216 (1.16).

The trait associations of 16 pod physical traits are depicted in Table 2. SHS was negatively correlated with PT (r = -0.698) followed by VDR (r = -0.468), and positively

Table 1. Descriptive statistics for 16 pod physical traits in 254 genotypes in common bean. PL - pod length, PB - pod breadth, PT - pod thickness, LBR - length/breadth ratio, BTR - breadth/thickness ratio, DPL - dorsal pod length, VPL - ventral pod length, VDR - ventral/dorsal ratio, FPM - filled pod mass, SM - seed mass, PWM - pod wall mass, PW - pod wall, SPR - seed pod ratio, PWT - pod wall thickness, SP % - string, SHS - shattering score, CV - coefficient of variation.

Traits	Minimum	Maximum	Mean ± SE	CV [%]
PL [cm]	7.50	20.00	13.38 ± 0.13	15.41
PB [cm]	0.50	1.03	7.87 ± 0.07	13.10
PT [cm]	0.48	0.81	0.58 ± 0.42	10.95
LBR	9.76	28.17	15.20 ± 0.17	15.53
BTR	0.92	2.04	1.54 ± 0.09	10.52
DPL [cm]	7.30	20.00	13.32 ± 0.12	14.71
VPL [cm]	6.50	18.87	12.58 ± 0.81	14.93
VDR	0.89	1.01	0.94 ± 0.00	25.17
FPM [g]	6.47	19.89	13.32 ± 0.13	15.43
SM [g]	1.00	9.40	5.33 ± 0.96	28.56
PWM [g]	0.09	14.78	7.98 ± 0.16	31.72
PW%	0.01	4.31	1.22 ± 0.04	32.24
SPR	0.13	0.97	0.73 ± 0.01	13.81
PWT [cm]	0.02	1.28	0.34 ± 0.01	33.81
SP	1.00	28.10	21.48 ± 0.90	30.46
SHS	1.07	9.13	6.09 ± 0.15	28.17

Fig. 1. Shattering response in resistant (*upper row*) and susceptible (*lower row*) genotypes.

Fig. 2. Response of mechanical shattering in resistant (*left*) and susceptible (*right*) genotypes.

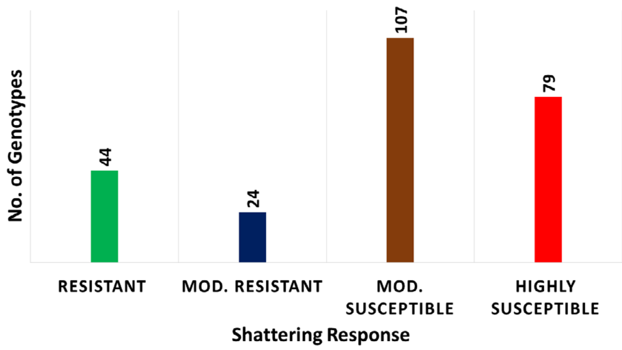

Fig. 3. Histogram showing shattering reaction of 254 bean genotypes.

correlated with various other pod physical traits including BTR (r = 0.599) followed by SP (r = 0.591), PB (0.185), and PL (0.177). There was no significant relationship of pod SHS with other pod physical traits. Among other traits PL was positively correlated with DPL (0.974), VPL (0.961), and FPM (0.981). PW% was positively correlated with PL (0.415). BTR was negatively correlated with PT (-0.679). SPR was positively correlated with seed mass (SM) (0.707) and negatively correlated with PWM (-0.527) and PW% (-0.576).

Principal component analysis: The PCA is a useful data reduction technique that helps plant breeder to reduce the data dimensions and exclude the traits that either have non-significant contribution towards variation or have non-significant correlation with the trait of interest. In the present study PCA was done based on 16 pod physical traits (Table 3) scored in the laboratory experiment. The number of PCA components was derived from correlation matrix and was equal to the number of traits. Based on the eigenvalue and the cumulative variance accounted by various components, the PCA concentrated 86.91% of variability in the first five principal components, for which the eigenvalue was greater than unity. The eigenvalue ranges from 5.93 for PC1 to 1.07 for PC5. Rest of the PCs were not considered as the eigenvalue was less than unity. The first two PCs that were used for biplot construction accounted for 55.62% of total variation (Table 4, Fig. 4).

Discussion

Variability for pod physical traits: Substantial variability in pod physical traits indicated significant diversity in common bean in respect of pod traits as depicted by broad ranges. Several earlier works have reported substantial diversity for pod length in landraces of beans ranging from 6.84 to 13.17 cm (Razvi *et al.* 2018), 7.67 to 26.33 cm (Sofi *et al.* 2020), 7.37 to 14.50 cm (Shama *et al.* 2022). Apart from its possible role in shattering behavior of pods, pod length, breadth, and thickness have great economic value in determining productive potential of common beans as it defines the yield of both snap and dry beans. The potential role of pod thickness is an important trait that may implicate pod shattering response (Tiwari and Bhatia

Table 2. *Pearson*'s correlation coefficient for 16 pod physical traits in common bean. For abbreviations *see* Table 1. * and ** indicate significance at 5 and 1% level of significance.

Variables	PL	PB	PT	LBR	BTR	DPL	VPL	VDR	FPM	SM	PWM	PW%	SPR	PWT	SP	SHS
PL	1	0.484**	-0.077	0.632**	0.403**	0.974**	0.961**	-0.050	0.981**	0.001	0.798**	0.415**	-0.139	0.035	0.094	0.177*
PB		1	0.007	-0.352**	0.722**	0.434**	0.391**	-0.263	0.486**	-0.010	0.401	0.204	-0.092	-0.020	0.189	0.185*
PT			1	-0.103	-0.679**	-0.058	-0.007	0.300*	-0.064	-0.031	-0.033	-0.039	-0.055	0.136	-0.201	-0.698**
LBR				1	-0.190	0.646**	0.664**	0.141	0.610**	0.012	0.489**	0.260	-0.063	0.066	-0.060	0.047
BTR					1	0.354*	0.291*	-0.379**	0.396*	0.019	0.310*	0.170	-0.028	-0.103	0.264	0.599**
DPL						1	0.986**	-0.041	0.989**	0.013	0.796**	0.410**	-0.133	0.042	0.078	0.131
VPL							1	0.123	0.978**	0.028	0.778**	0.391*	-0.117	0.039	0.053	0.053
VDR								1	-0.027	0.078	-0.068	-0.083	0.081	-0.040	-0.155	-0.468**
FPM									1	0.022	0.800**	0.405**	-0.126	0.018	0.089	0.154
SM										1	-0.583**	-0.826**	0.707**	0.009	0.023	-0.004
PWM											1	0.825**	-0.527**	0.009	0.058	0.128
PW%												1	-0.576**	0.004	0.049	0.088
SPR													1	-0.074	-0.072	0.027
PWT														1	-0.002	0.033
SP															1	0.591**
SHS																1

1995). Pod thickness is invariably higher in snap beans which are usually resistant to shattering. Pod length and wall thickness may play a role in pod shattering resistance (Krisnawati *et al.* 2019).

Similarly, length/breadth and breadth/thickness ratios are important traits in determining bean types. Pods with breadth/thickness ratio tending to unity can be in both pulse or snap type beans based on pod succulence, those with breadth/thickness ratio greater than unity are invariably pulse types and their seeds are invariably flatter. Similarly, pods with breadth/thickness ratio less than unity are invariably snap beans with succulent pods. They are usually resistant to shattering. Similar results have been reported in common bean by Bozoglu and Sozen (2011). The dorsal and ventral length of pods and their relative ratios determine the curvature of pods that has significant effect on pod shattering due to differential pressures developed on both sutures upon pod drying and as such are important parameters in shattering response. The ventral/dorsal ratio indicates the curvature of pods and is correlated with pod shattering (Sofi *et al.* 2022). Tsuchiya (1987) in soybean found that resistant genotypes had higher curvature (lower VPL/DPL ratio) as compared to susceptible genotypes (higher VPL/DPL ratio).

Pods shatter due to highly fibrous and parchment pod walls compared to pods with less fiber that are less vulnerable to shattering (Gioia *et al.* 2013). In the classical example of snap beans, secondary domestication resulted in completely indehiscent pods, especially in the Andean gene pool (Wallace *et al.* 2018), making them more suitable for consumption as green pods due to the low fiber content in the pod walls and sutures (stringless bean). Similarly, traits such as pod wall thickness and pod length may play key role as determinant factors in pod shattering resistance. Therefore, resistance to pod shattering could be enhanced by increasing the thickness of the pod wall (Krishnawati and Adie 2016). Since the degree of coiling of pod walls is strongly influenced by thickness of the wall fiber layer (Takahashi *et al.* 2020), the increased pod wall fiber thickness leads to yield penalties by promoting pod shattering as well as competing with seeds for photosynthates (Assefa *et al.* 2013). The range of trait dispersion is depicted by range and CV value showed that highest CV value was observed in case of pod wall thickness (33.81%) followed by pod wall% (32.24%), pod wall mass (31.72%), string proportion (30.46%), while the lowest value of CV was observed in breadth/thickness ratio (10.52%).

Genetic variability for shattering response: Pod shattering score had a broad range from almost resistant (SS = 0) to completely shattered (SS = 10). Several researchers have reported significant variation for shattering score in various model legume species. Guo *et al.* (2022) reported wide variation of pod shattering score in *Medicago* with average shattering of 76%. However, among resistant types, shattering score was only 7.4%. Krisnawati and Adie (2017) reported highly significant difference for pod shattering in soybean, indicating the high variation in shattering resistance

Table 3. Latent scores (eigenvalues) for 16 pod physical traits in common bean.

Component	Eigenvalue	Variability [%]	Cumulative variance [%]
PC1	5.93	37.12	37.12
PC2	2.96	18.50	55.62
PC3	2.46	15.39	71.01
PC4	1.47	9.20	80.21
PC5	1.07	6.70	86.91

Table 4. Trait contributions to PCs (factor loadings) for 16 pod physical traits in common bean. For abbreviations *see* Table 1.

Trait	PC1	PC2	PC3	PC4	PC5
PL	0.387	0.090	0.161	0.026	-0.023
PB	0.217	0.189	-0.050	0.634	-0.063
PT	0.830	-0.419	-0.088	-0.304	-0.234
LBR	0.224	-0.253	-0.132	-0.541	0.016
BTR	0.089	0.422	-0.100	0.216	0.117
DPL	0.393	-0.116	-0.166	0.006	0.029
VPL	0.371	-0.174	-0.176	0.006	0.008
VDR	-0.062	-0.359	-0.059	-0.011	0.251
FPM	0.386	-0.098	0.172	0.044	-0.006
SM	-0.103	-0.002	-0.597	0.040	-0.076
PWM	0.376	-0.082	0.220	0.011	0.041
PW%	0.255	-0.031	0.437	-0.039	0.076
SPR	-0.133	0.037	-0.487	-0.007	0.049
PWT	0.011	-0.051	-0.020	-0.077	-0.905
SP	0.111	0.370	-0.084	-0.187	-0.145
SHS	0.119	0.454	-0.060	-0.279	-0.076

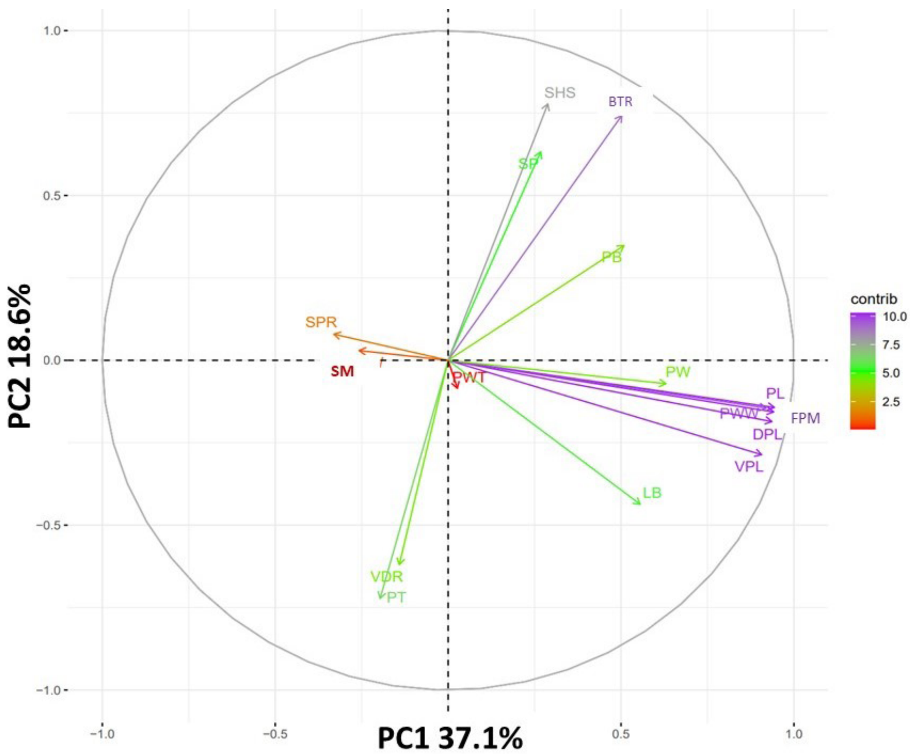

Fig. 4. PCA biplot on the basis of PC1 and PC2.

among genotypes. Similarly, Murgia *et al.* (2017) also reported wide variation in a common bean diversity in panel of 267 genotypes comprising a susceptible (MG38) and resistant (MIDAS) cultivars and their introgression lines. They found significant variation in level as well as mode of shattering and reported that pod shattering was more pronounced in lines with smaller pods, lower pod mass, and pods with lower seed to pod ratio, indicating a significant metabolic cost of pod shattering, much enough to limit the size of seed. The 100-seed mass of shattering resistant types in adzuki bean (*Vigna angularis*) and yard long bean (*Vigna unguiculata* cv. *sesquipedalis*) has also been found to be higher than in the wild types (Takahashi *et al.* 2020). Similarly, the degree of coiling of pod walls also has a strong positive relationship with the pod wall thickness (Takahashi *et al.* 2020). Therefore, these traits can be used in screening for pod shattering in common bean and other legumes. In terms of shattering response, out of 254 accessions, 44 were resistant (shattering score of 1 - 3), 24 were moderately resistant (shattering score of 4 - 5), 107 were moderately susceptible (shattering score of 6 - 7), and 79 were highly susceptible (shattering score of 8 - 10) (Fig. 3). Murgia *et al*. (2017) also reported similar distribution in a panel of 267 introgression lines derived from MG38 × MIDAS, out of which 29 lines were completely resistant to shattering and only 10% of lines showed complete shattering resistance greater than MIDAS. 15% of lines showed shattering susceptibility greater than MG38. In terms of variability, contrasting genotypes WB-1129 and WB-216 (resistant) and WB-6 and WB-371 (susceptible) were also subject to uniform manual twisting and there was obvious difference in pattern of pod shattering response (Fig. 2).

Trait association depicting relationship between physical traits and shattering: Among pod physical traits the important traits that drive shattering resistance are pod thickness and ventral/dorsal ratio. The pods with smaller VDR (higher curvature) are more susceptible to shattering as the curvature compounds the pressure exerted by pod walls on the pod suture and promotes pod breakdown as well as twisting. Similarly, the thicker pods are invariably succulent and have thicker pod walls and contain higher amounts of starch, cellulose, pectin, and lignin that improve shattering. Similar results have been reported by Krisnawati *et al.* (2019, 2020) in soybean. Seed to pod ratio was positively correlated with seed mass (0.707) and negatively correlated with pod wall mass (-0.527) and pod wall% (-0.576). Tsuchiya (1987) found that traits like length, breadth, and thickness as well as pod curvature are important determinants of pod shattering in soybean. Murgia *et al*. (2017) found significant variation in level and mode of shattering and reported that pod shattering was more pronounced in lines with smaller pods, lower pod mass, and pods with lower seed to pod ratio. Kataliko *et al.* (2019) reported that pod shattering resistance in soybean was negatively correlated with a number of seeds per pod and plants with few seeds per pod (smaller pods) tended to have high resistance to pod shattering. Similarly, the degree of coiling of pod walls also has a strong positive relationship with the pod wall thickness (Takahashi *et al.* 2020). Therefore, these traits can be used in screening for pod shattering in common bean and other legumes.

Invariably in legumes, the relative values of pod thickness and width and their ratio has been considered as a critical trait during dehiscence (Caviness 1969, Zhang *et al.* 2018). In addition, fruit length and curvature have been also found to be important morphological traits related to shattering response. However, a contrary observation has been reported in *Lotus conimbricensis,* where the indehiscent pods were strongly curved (Grant 1996). Similarly, other studies (Suzuki *et al.* 2009, Dong *et al.* 2017) observed that there was no significant correlation of shattering response with length, width, and thickness of pods and also the thickness/width ratio was not associated with pod shattering. Even though pod wall thickness has been found to be significantly correlated with shattering in soybean (Tiwari and Bhatia 1995), we could not observe any such relationship in common beans.

Principal component analysis: The GT biplot constructed based on PC1 and PC2 outlines the relationship between traits, elucidated by the angle of traits with the target trait as well as the length of trait arrow. In terms of biplot pod shattering score was significantly and positively correlated with breadth/thickness ratio, string %, and pod breadth, but negatively correlated with pod thickness and ventral/dorsal ratio. The results are fairly in agreement with the correlation analysis even though sometimes the relationship based on PCA biplot is different than correlation analysis as it captures only a part of variation (55.62% in present case). There are no reports of multivariate analysis of pod physical traits in relation to pod shattering in common bean. However, Tu *et al.* (2019) used principal component analysis among various anatomical traits of ventral suture in soybean in relation to pod shattering and reported that first two axes explained 93.6% of the total variance in the shatter-susceptible and three shatter-resistant soybean cultivars.

Conclusion

Pod physical traits are important determinants of shattering response. In the present study, we screened a set of 254 genotypes using pod physical traits to identify effective surrogates for improving shattering resistance. We identified pod thickness, ventral/dorsal pod ratio, string %, breadth/thickness ratio, and pod length as important drivers of shattering response. Random Impact Assessment is a useful approach for assessing large scale germplasm evaluation for traits like shattering, whose screening under field conditions is implicated by weather changes as well as moisture status of pods. The method creates a fairly uniform screening system for shattering response and removes all subjectivities. We identified several shattering resistant genotypes that have been used in crossing program to develop mapping populations for molecular characterization of pod shattering. The resistant lines have also been registered with National Gene Bank of India for long term storage and conservation. As for the ideal trait combinations for improving shattering response breeders should select for relatively shorter, thicker, and straighter pods. However, we should also look for stringless type pods as the increased fiber content not only increases susceptibility but also has yield penalties. In our studies also the resistant genotypes WB-216 and WB-1129 had straighter and thicker pods whereas the susceptible genotypes WB-371 and WB-6 had curved pods with greater dorsal length and thin papery pods.

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