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Identification of resistant sources for pod shattering in a cowpea (*Vigna unguiculata* L.) core collection using a modified screening system based on weighted level scores using random impact method

Research Article

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Corresponding author:
Parvaze A. Sofi;
Email: parvazesofi@gmail.com

Deepak Bijarniya[1], Sadiah Shafi[1], Aaqif Zaffar[1], Ishrat Riyaz[1], Samreen Fatima[1], Sajad Majeed Zargar[2], Kuldeep Tripathi[3], P. V. Vara Prasad[4] and Parvaze A. Sofi[1]

[1]Stress Physiology Lab, Division of Genetics and Plant Breeding, SKUAST-Kashmir, Wadura, India; [2]Division of Plant Biotechnology, SKUAST-Kashmir, Shalimar Campus, India; [3]Division of Germplasm Evaluation, ICAR-NBPGR, New Delhi, India and [4]Sustainable Intensification Innovation Lab, Kansas State University, Manhattan, KS, USA

Abstract

In the present study we evaluated a core set of 254 cowpea genotypes for seven pod physical traits and shattering score using a modified weighted average screening system based on random impact assessment. There was substantial variability in all the pod physical traits and shattering score in the cowpea core collection indicating significant diversity of the material in respect of pod traits. Shattering score had a mean value of 5.39 with a range of 0–10. Out of 254 genotypes, 34 were resistant, 83 were moderately resistant, 82 were moderately susceptible and 55 were highly susceptible. Shattering score had significant negative correlation with pod length followed by pod weight, pod breadth, seeds per pod, pod wall weight and pod thickness. PCA concentrated 69.60% variability in the first two principal components with Eigen value of 4.49 for PC1 and 1.07 for PC2, mainly contributed by pod weight, pod length, pod breadth, pod thickness and pod wall weight. The conventional screening methods are based on level of shattering and do not take into account various types of shattering such as fissured, split, twisted or abscised. The present was aimed at identification of shattering resistant genotypes using a modified screening method based on weighted level averages. The study identified several genotypes highly resistant to pod shattering that can be used to develop shattering resistant cowpea varieties for sustainable cowpea farming and highlights the effectiveness of proposed screening method.

Introduction

Cowpea (*Vigna unguiculata* L.) is one of the most important legume crops that is fairly adapted to harsher environments especially drought and high temperature. In India it is used as a dual-purpose crop used for food and fodder. In the North-Western Himalayan region especially Kashmir valley it is a niche crop that fetches premium price on account of its broader adaptability and nutritional value viz. high protein content as well as its ability to fix nitrogen. However, a number of production constraints such as pod shattering severely implicate its yield potential especially under changing climate with drier and warmer days around maturity period of the crop. Cowpea was domesticated in Africa from its wild ancestor *V. unguiculata* var. *dekindtiana*) some 5000–6000 years ago (Coulibaly *et al.*, 2002). The loss of yield from pod shattering can be either on account of the pre-harvest pod shattering in the field, and the yield loss from pod shattering during manual or mechanical stress. This problem is exacerbated in dry arid environments where pods become brittle and fracture easily. There is no published information available in cowpea in terms of losses caused by shattering, but losses as high as 20–60% have been reported in soybean, broad bean, brassica and canola (Tiwari and Bhatnagar, 1991; Price *et al.*, 1996; Child *et al.*, 1998; Dong *et al.*, 2016). Pod shattering also has a metabolic cost as it limits the seed size (Murgia *et al.*, 2017). In fact, 100-seed weight of shattering resistant types in *V. unguiculata* subsp. *sesquipedalis* was reported to be higher than the wild types (Takahashi *et al.*, 2019). The domesticated cowpea has fairly lesser level of pod shattering, despite the fact that the level of shattering in cultivated germplasm is fairly high with persistence of shattering in some varieties. As such shattering assumes significant importance as a cause of yield losses especially under dry hot environments (Zhang and Singh, 2020; Parker *et al.*, 2021). Therefore, there is an urgent need to develop shattering resistant varieties to avoid such losses.

Despite the fact that human selection under domestication of crops has driven selection of desired plant traits including adaptive traits such as reduced shattering, plants have still retained fair amount of seed shattering to provide for ease of harvesting (Gregory and Ellis, 2009; Flint-Garcia, 2013). In wild plants, shattering is desirable as it allows seed dispersal, but in commercial agriculture, it is undesirable as it leads to substantial loss of yields. Pod shattering is the most important domestication syndrome that has evolved independently in legume crops during the domestication process, and has driven adaptation of the legumes plants to diverse agro-ecosystem (Tang *et al*., 2013).

In cowpea, the major domestication driven changes have progressively reduced pod dehiscence and seed hardness. Also, there has been an increase in size of pods and seeds by increased rate of dry weight accumulation (Lush and Evans, 1981; Andargie *et al*., 2014). Shattering of pods is mainly governed by hygroscopic movements within the pod valves upon dehydration (Lo *et al*., 2018). The accumulation of tension during dehydration causes splitting of the valves along their suture lines (Elbaum and Abraham, 2014). Shattering invariably occurs at the final stages of pod development and is triggered by senescence and desiccation of lignified cells in the pods (Roberts *et al*., 2002). In cowpea, pod shattering has been found to be influenced by the thickness and strength of the fibre layer of the pod that generates twisting force (Takahashi *et al*., 2020).

Several pod physical traits such as pod wall weight and pod wall water content, pod number, thickness of the pod wall, and seed weight/pod weight ratio, pod thickness, pod length have been implicated in shattering response with most of the work reported from soybean and common bean (Kuai *et al*., 2016; Krisnawati and Adie, 2017; Zhang *et al*., 2018; Krisnawati *et al*., 2020; Sofi *et al*., 2022). The present study was the first comprehensive phenotyping study in Western Himalayan region using national core set of cowpea for pod shattering in relation to various pod physical traits with the broader aim of identifying sources of resistance to shattering using a modified screening method that is based on weighted level scores. The broad hypothetical framework of present study was that the pod shattering has a definite physical basis in terms of being affected by pod physical traits.

Materials and methods

Site of the experiment

The experiment was conducted in 2022 and 2023 at the research fields of Division of Genetics and Plant Breeding, Faculty of Agriculture Wadura, SKUAST-K, Sopore (34°17′ North and 74°33′ E at an altitude of 1594 masl). The experimental site soil is a typical inceptisol with predominantly clay loam texture. The pH is almost neutral (7.2), with organic carbon 0.65%, electrical conductivity of 0.18 dS/m and CEC of 16 meq/kg. All the accessions were grown as single rows of four-meter length, with a spacing of 20 cm × 60 cm, in an augmented block design with four checks. The minimum and maximum temperatures during the growing season in 2022 varied from 10.50–17.91 and 26–32°C and in 2023 it ranged from 9.10–18.01 and 23.2–33.50°C, respectively. The total precipitation during the crop period in 2022 and 2023 was 194.60 and 388.40 mm respectively. Data was recorded on ten competitive plants from each genotype for pod length, pod breadth, pod thickness, seeds per pod, 100-seed weight, pod weight, pod wall weight and shattering score as per the standard procedures (Sofi, 2019; Fatima *et al*., 2023).

Experimental material

The material for the present study comprised of a core set of 248 lines from national cowpea core set developed by NBPGR (National Bureau of Plant Genetic Resources) including six checks (Shalimar cowpea-1, Shalimar cowpea-2, Local Bold-1, WFC-1, WFC-2 and WFC-3), representing diverse market classes in cowpea. The core set comprised of a vast diversity of growth habits, seed colour, shape and eye pattern. All the accessions were grown as single replicates in an augmented block design (ABD) with a 2.5 m row with spacing of 20 cm within rows and 60 cm between rows, except the checks that were replicated in each block. Each block of the ABD comprised of 31 accessions and six checks.

Crop management

The management practices were uniform and homogeneous and comprised of seed treatment with the fungicide (Captan 50WP) and the insecticide (Imidacloprid 17.8%) 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 top dressing 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.

Pod physical traits recorded in the present study

Pod length (cm): Average length of pods was calculated in cm using a measuring scale from base of pod to the tip except the beak if any

Pod breadth (cm): Average breadth of pods was calculated in mm using vernier calliper

Pod thickness (cm): Average thickness of pods in mm using vernier calliper

Seeds per pod: Number of seeds per pod averaged over 10 pods

100-seed weight (g): Weight of 100 seeds averaged over three randomly drawn samples.

Pod weight (g): Average weight of pods of including seeds using weighing balance in grams

Pod wall weight (mg): Average weight of pods excluding seeds using weighing balance in grams

Shattering score: Weighted average of number of pods in various types of shattering

Manual screening for pod shattering using random impact method

Field phenotyping of pod shattering requires fully grown plants, and it is a time-consuming and labour-intensive procedure (Kim *et al*., 2020). Moreover, the fluctuations in weather parameters at the time of pod maturation cause bias in the results. However, a test procedure, namely Random Impact Apparatus (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). In the present study, screening of pod shattering was done in laboratory using RIM 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. Random impact method (RIM) enables the rapid comparison of shattering

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response of fully mature pods from individual plants (Bruce *et al.*, 2002). The pods were harvested at physiological maturity and were equilibrated for moisture for 10 d in paper bags (20 × 10 cm) followed by oven drying at 80° for 2 d. Ten sampled pods were put in RIA and shaken on a shaker at 250 rpm for 10 s using a stopwatch. Each treatment was done in triplicate. The method subjects all samples to uniform conditions as well as manual pressure. In addition, Murgia *et al.* (2017) observed that almost 98% of the variance for shattering was genetic and there was a very limited role of environmental factors influencing this trait.

Development of a modified screening index

We developed a modified screening index following the protocol for level of shattering as proposed by Murgia *et al.* (2017). In the conventional method, the shattering score is usually estimated as percentage of shattered pods from a given sample, without any delineation of type of shattering. In this study, we measured the shattering response of pods in terms of 1–9 scale where 1 = indehiscent pods, 2 = fissured pods, 3 = split pods, 4 = split pods with 20% twist, 5 = split pods with 40% twist, 6 = split pods with 60% twist, 7 = split pods with 80% twist, 8 = split pods with 100% twist, and 9 = Abscised pods. In order to build the shattering score index, the number of pods in each *ith* class (type of shattering) was multiplied by the *ith* scale (numerical description of the scale) and the final shattering score (SS) was taken as weighted average of all the values as follows:

$$\mathrm{SS} = 1/n \sum_{n=1}^{i} (\text{Number of pods in a scale} \times \text{Scale value})$$

Results

Variability for pod physical traits and shattering score

There was substantial variability in seven pod physical traits and shattering score in 254 genotypes of cowpea core collection indicating significant diversity of the material in respect of pod traits (Table 1, online Supplementary Figs. S1–S4). Pod length had a mean value of 15.56 cm (8.00–31.67 cm). Highest pod length was recorded in case IC471926 (31.67 cm), followed by IC58905 (28.40 cm) and WFC-3 (23.67 cm), whereas IC202710 has smallest pods measuring 8.00 cm. Pod breadth had a mean value of 7.44 mm (4.23–12.20 mm). Highest pod breadth was recorded in case EC724805 (12.20 mm), followed by EC723822 (10.97 mm) and EC724239 (10.80 mm), whereas pod breadth was smallest in case of IC397618 (4.23 mm). Pod thickness had a mean value of 6.06 mm with a range of 4.10–9.67 mm. Highest pod thickness was recorded in case IC413324 (9.67 mm), followed by EC244130 (9.20 mm) and EC724805 (9.10 mm), whereas pod thickness was smallest in case of IC383461 (4.10 mm). Pod weight had a mean value of 2.43 g with a range of 0.63 to 4.73 grams. Highest pod weight was recorded in case IC282020 (4.73 g), followed by IC413324 (4.52 g) and EC244025 (4.42 g) while as lowest value for pod weight was recorded in IC202710 (0.63 g). Pod wall weight had a mean value of 649.74 mg (136.00 to 2103 mg). Highest pod wall weight was recorded in case IC282020 (2103.00 mg), followed by EC725103 (1506.00 mg) and EC724319 (1504.00 mg) while as lowest value for pod wall weight was recorded in IC397618 (136.00 mg). Seeds per pod had a mean value of 13.67 with a range of 7.67–21.00. Highest number of seeds per pod were recorded in case of EC98661 (21.00), followed by EC98678 (20.67) and IC97807 (20.00), whereas the lowest number of seeds per pod were recorded in case of EC16205 and IC372722 (7.67). Similarly, for 100-seed weight a broader range of 3.80–30.82 g was observed with the highest 100-seed weight recorded in case of C-196 (30.82 g), followed by IC336836 (29.48 g) and EC18734 (28.00 g), whereas lowest 100-seed weight was recorded for EC738277 (3.80 g).

There was substantial variability in pod shattering score in 254 genotypes of cowpea indicating significant diversity of the material in respect of shattering response (Fig. 1). Using the conventional scoring method of shattering response based on percentage of sampled pods shattered, regardless of the type of shattering, out of 254 genotypes, 18 were highly resistant, 22 were moderately resistant, 40 were moderately susceptible and 178 were highly susceptible. Shattering score had a mean value of 5.39 with a range of 1.00 in resistant genotypes to 10.00 in highly susceptible genotypes. Highest shattering score of 10.00, that indicates highest susceptibility to shattering, was recorded in case EC738122 and EC738159, followed by a score of 9.78 recorded for EC7511 and EC528687. One accession with a shattering score of 1 viz., EC332352 was completely indehiscent even under manual shattering force, followed by highly resistant accessions IC58905, IC413324 and IC361790 with a score of 1.11. Out of 254 genotypes, 34 were resistant (SS = 0–2.5), 83 were moderately resistant (2.51–5), 82 were moderately susceptible (5.1–7.5) and 55 were highly susceptible (SS = 7.51–10).

Trait association among pod physical traits in cowpea core set

The trait associations of eight pod physical traits are depicted in Fig. 2. Shattering score had significant negative correlation with pod length ($r = -0.513$) followed by pod weight ($r = -0.510$), pod breadth ($r = -0.440$), seeds per pod ($r = -0.420$), pod wall weight ($r = -0.410$) and pod thickness ($r = -0.383$). Among other pod traits, significant positive correlations were recorded with notable correlations between pod breadth and pod weight ($r = 0.830$), followed by pod length and pod weight ($r = 0.810$), pod weight and pod thickness ($r = 0.770$), pod weight and pod wall weight ($r = 0.760$), pod breadth and pod thickness ($r = 0.740$), pod length and pod breadth ($r = 0.700$), pod length and

Table 1. Descriptive statistics of pod physical traits in cowpea core collection

Parameter	PL	PB	PT	SPP	100SW	PW	PWW	SS
Mean	15.56 ± 0.21	7.44 ± 0.09	2.43 ± 0.06	13.76 ± 0.16	15.15 ± 0.32	2.43 ± 0.06	649.74 ± 18.50	5.39 ± 0.15
Minimum	8.00	4.23	0.63	7.67	3.80	0.63	136.00	0.00
Maximum	31.67	12.20	4.73	21.00	30.82	4.73	2103.00	10.00
CV (%)	21.80	18.90	26.60	18.20	23.80	26.60	35.40	34.40

PW, Pod weight; PL, Pod length; PB, Pod breadth; PT, Pod thickness; SPP, Seeds per pod; 100SW, 100 seed weight; PWW, Pod wall weight; SS, Shattering score.

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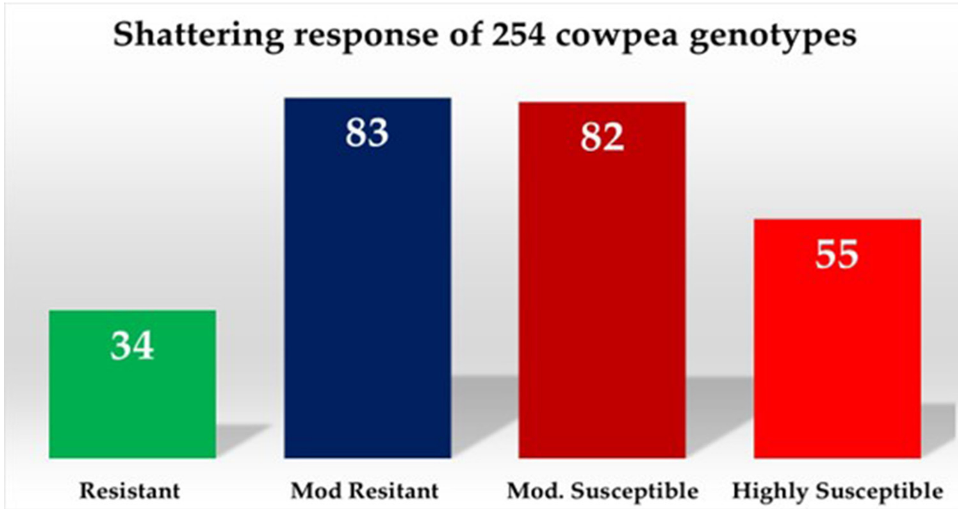

Figure 1. Response of 254 cowpea genotypes to manual shattering based on modified screening system.

pod wall weight ($r = 0.640$), pod breadth and pod thickness with pod wall weight ($r = 0.620$). There were no significant correlations recorded among pod traits except with shattering score.

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 eight pod physical traits (Table 2) scored in the cowpea core set. The number of PCA was derived from correlation matrix and is equal to the number of traits. Based on the Eigen value and the cumulative variance accounted for the PCA, the PCA concentrated 69.60% variability in the first two principal components which were significant based on Eigen values greater than unity. The Eigen value was 4.497 for PC1 and 1.07 for PC2. Rest of the PCs were

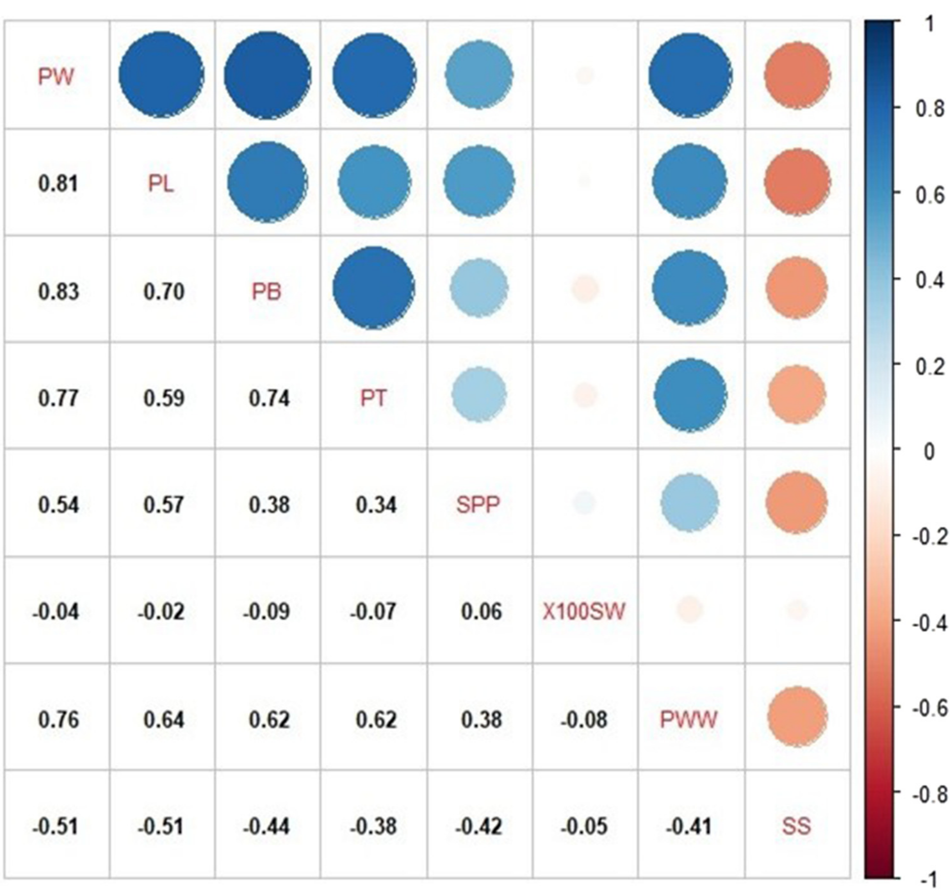

Figure 2. Heat map of pod physical traits in cowpea, Where, PW, Pod weight; PL, Pod length; PB, Pod breadth; PT, Pod thickness; SPP, Seeds per pod; 100SW, 100 seed weight; PWW, Pod wall weight; SS, Shattering score.

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Table 2. Eigen values and variances accounted by principal components

Component	Eigen value	Per cent variance accounted	Cumulative variance accounted
PC1	4.497	56.2	56.20
PC2	1.074	13.4	69.60

not considered as the Eigen value was less than unity. The first two PC's were used for construction of biplot that accounted for 69.60% of total variation (Fig. 3). The traits that significantly contributed to the PC1 were pod weight, followed by pod length, pod breadth, pod thickness and pod wall weight. The PC2 was largely contributed by 100-seed weight (Table 3).

Discussion

In the present study substantial variability in pod physical traits in the cowpea core set was observed in respect of pod length, pod breadth, pod thickness, pod weight and pod wall weight as well as shattering score. Several earlier works have reported substantial diversity for pod length in cowpea ranging from 12.3–74.5 cm (Xu *et al*., 2017), 10.44–20.12 cm (Sofi *et al*., 2022), 7.50–20.00 cm (Fatima, 2023). Pod length, breadth and pod wall weight have significant role in shattering response (Krisnawati and Adie, 2017). Pods with greater pod wall weight (thicker pods) are fairly resistant to shattering (Gioia *et al*., 2013; Fatima, 2023) as the degree of pod coiling of pod walls is strongly influenced by thickness of the wall (Krisnawati and Adie, 2017; Takahashi *et al*., 2019). The range of trait dispersion as depicted by range and C.V. value showed that highest C.V. value was observed in case of pod wall weight (35.40%) followed by shattering score (34.40%), pod weight and pod thickness (26.60%), 100-seed weight (23.80%) and pod length (21.80%), while as the lowest value of C.V. was observed in seeds per pod (18.20%).

Pod shattering score had a broad range from almost resistant (SS = 0) to completely shattered (SS = 10). Out of 254 genotypes, 34 were resistant (SS = 0–2.5), 83 were moderately resistant (SS = 2.51–5), 82 were moderately susceptible (5.1–7.5) and 55 were highly susceptible (SS = 7.51–10). Several earlier workers have reported substantial variation for shattering score in several legume species. Guo *et al*. (2022) has reported wide variation of

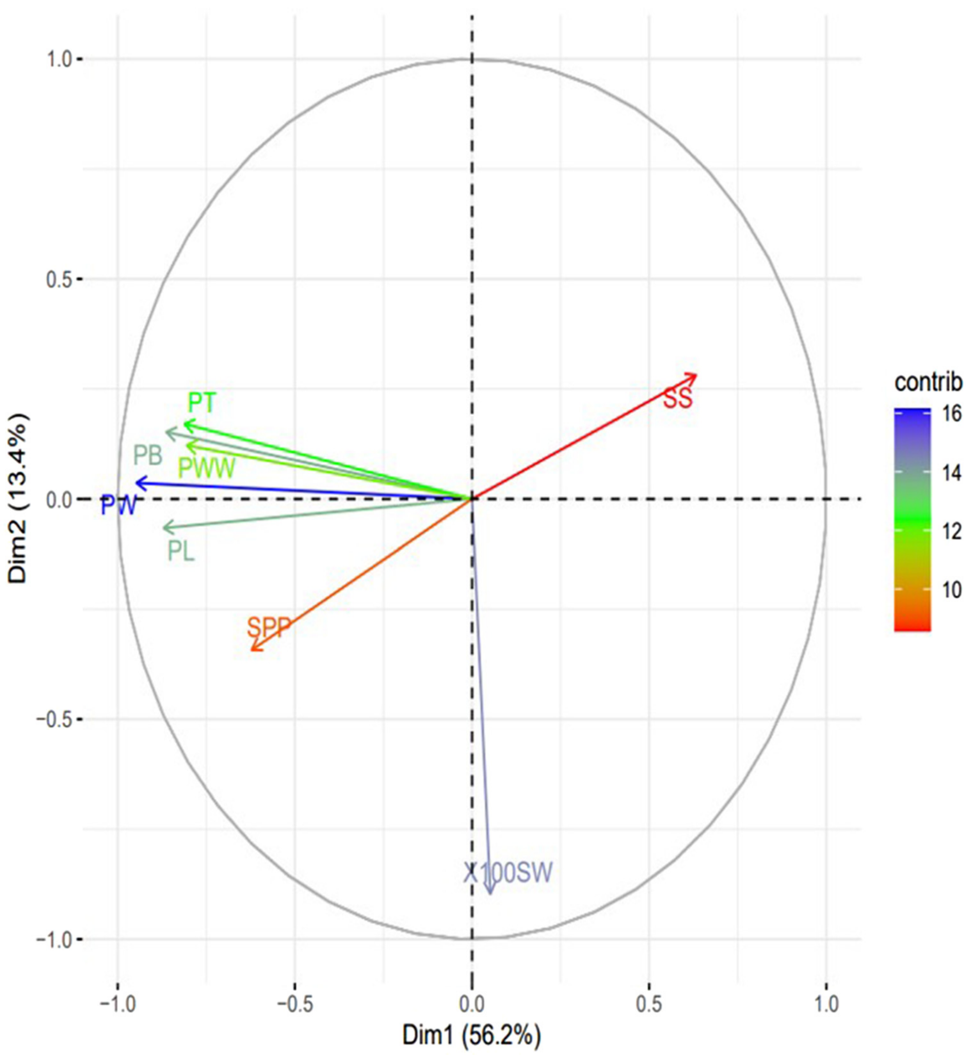

Figure 3. PCA biplot of various pod physical traits, Where, PW, od weight; PL, Pod length; PB, Pod breadth; PT, Pod thickness; SPP, Seeds per pod; 100SW, 100 seed weight; PWW, Pod wall weight; SS, Shattering score.

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Table 3. Component loadings of pod physical traits

Trait	PC1	PC2
PW	0.956	NS
PL	0.859	NS
PB	0.894	NS
PT	0.846	NS
SPP	0.556	NS
100SW	NS	0.915
PWW	0.830	NS
SS	−0.578	NS

PW, Pod weight; PL, Pod length; PB, Pod breadth; PT, Pod thickness; SPP, Seeds per pod; 100SW, 100 seed weight; PWW, Pod wall weight; SS, Shattering score.

pod shattering score in *Medicago* ranging from 7.4–76%. Krisnawati and Adie (2017) reported highly significant difference for pod shattering in soybean, indicated the high variation in shattering resistance among genotypes. Similarly, Murgia *et al.* (2017) has also reported wide variation in a common bean diversity panel of 267 genotypes comprising a susceptible (MG38) and resistant (MIDAS) and their introgression lines with shattering percentage of 0–82.6% with a mean of 31.6%. Kaur *et al.* (2020) reported that black mustard was more susceptible to shattering as compared to Indian mustard and rapeseed and reported significant variation in terms of rupture energy. In our earlier studies on common bean pod shattering, Fatima *et al.* (2023) reported shattering score ranging from 0–10 with a mean of 6.098 in a common bean diversity panel. However, all these studies were based on conventional method of pod shattering screening without any distinction to different types of shattering.

The trait association revealed that shattering score had significant negative correlation with pod length followed by pod weight, pod breadth, seeds per pod, pod wall weight and pod thickness. Based on these trait associations it can be premised that pod length, pod weight, pod breadth, seeds per pod, pod wall weight and pod thickness are major drivers of shattering resistance in cowpea. Thus, longer pods with wider diameter and thicker pod wall (resulting in higher pod wall weight) are more resistant to shattering. Such pods have pod walls containing higher amounts of starch, cellulose, pectin and lignin that improve shattering (Fatima *et al.*, 2023). Similar results have been reported by Krisnawati *et al.* (2019, 2020) in soybean and Fatima *et al.* (2023) in common bean. In fact, Krisnawati *et al.* (2020) reported highly significant negative correlation between pod shattering and seeds per pod in soybean, but shattering had positive correlation with dorsal length of pods that indicates greater curvature of pods. Murgia *et al.* (2017) also reported more pronounced pod shattering in lines with smaller pods, lower pod weight and pods with lower seed to pod ratio. The greater influence of pod length on shattering response in cowpea might be on account of parallel changes in in cowpea under domestication viz., increased strength of dehiscence zone in pod sutures, increase in size of pods and seeds as well as reduction in dehiscence (Lush and Evans, 1981). Similarly, Kataliko *et al.* (2019) reported that pod shattering resistance in soybean was negatively correlated with number of seeds per pod. The degree of pod coiling has a strong positive relationship with the pod wall thickness as reported by Takahashi *et al.* (2020). However, some workers such as Suzuki *et al.* (2009) and Dong *et al.* (2017) reported that there was no significant correlation of shattering response with length, width, and thickness of pods and the thickness: width ratio was not associated with pod shattering.

The PCA concentrated 69.60% variability in the first two principal components which were significant based on Eigen values greater than unity. The traits that significantly contributed to the PC1 were pod weight, followed by pod length, pod breadth, pod thickness and pod wall weight. The PC2 was largely contributed by 100-seed weight.

In terms of GT biplot pod shattering score was significantly and negatively correlated with pod length, pod weight, pod breadth, seeds per pod, pod wall weight and pod thickness. The results are fairly in agreement with the correlation analysis even though sometimes the relationship based on PCA biplot are different than correlation analysis as it captures only a part of variation (69.60% in present case). This is the first report of multivariate analysis of pod physical traits in relation to pod shattering in cowpea. 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. Similarly, Fatima (2023) reported in common bean diversity panel that first two PC's accounted for 50.74% of variation largely contributed by pod wall thickness, breadth thickness ratio, string per cent, length breadth ratio, pod length, pod breadth, pod thickness, ventral pod length and filled pod weight.

The conventional screening methods as proposed by Murgia *et al.* (2017) and one reported by Fatima (2023) are efficient in delineating genotyping differences for pod shattering on the basis of number of pods shattered out of the sampled pods subjected to manual shattering pressure using a RIM apparatus. However, it is based on level of shattering and does not take into account various types of shattering such as fissured, split, twisted or abscised (online Supplementary Fig. S3). It has been observed that in several cases, fissured and partially split pods do not lead to complete loss of seed as compared to twisted and abscised pods. In order to quantify the relative contribution of each type of shattering response within sampled pods, we used an average of weighted level scores as described above. The method takes into account the number of pods in a sampled set from each class of shattering to derive the shattering score that is more reliable than earlier methods. We propose that this method should be used for screening shattering response under manual pressure methods to overcome the ambiguity of conventional methods. It creates fairly uniform conditions for pods as they are equilibrated for moisture and subjected to similar manual pressure.

Conclusion

The present study is the first comprehensive screening report of a large-scale germplasm evaluation for pod shattering. We used Random Impact Assessment, which is a useful approach for characterising large germplasm collections for traits like shattering, whose screening under filed 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 important pod physical attributes that are important determinants of shattering response in cowpea. In the present study, we screened a set of 254 cowpea genotypes of national core collection using pod physical traits to identify effective traits for improving shattering resistance. We identified pod length, pod weight, pod breadth, seeds per pod, pod wall weight and pod thickness as important drivers of

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shattering response in cowpea. We identified several shattering resistant genotypes (EC332352, IC471926, WFC-3, IC58905, IC413324 and IC361790, EC109493-2744-1, EC109493-1047-1, EC738109 and EC724319) that can be used in crossing programme to develop mapping populations for molecular characterization of pod shattering. As for the ideal trait combinations for improving shattering response breeders should select for relatively longer, thicker and straighter pods. The findings of the study are useful not only from the point of view of identifying potential sources of resistance to shattering, but can also be used to map genetic loci underlying shattering response in cowpea.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262124000388.

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Competing interests. The authors declare they have no conflicts of interest.

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