

Insights into the biochemical basis of pod shattering in common bean (*Phaseolus vulgaris* L.) from Western Himalayas

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ABSTRACT

Pod shattering is the one of the important domestication syndromes. From an agricultural perspective, it is generally an undesirable process, and in common beans, pre-harvest shattering loss is the major form of yield loss by shattering. Thus, deciphering the physical, biochemical and genetic basis of pod shattering is important to unravel the mechanisms of parallel evolution and also because this will provide breeders with key information to manipulate this trait to reduce yield loss. There are only a few published records are available in common bean. In the present study, a core set of 254 lines were evaluated for shattering response using Random Impact Apparatus. Based on the shattering response using a random impact assessment 40 contrasting lines were selected for biochemical analysis. Pod lignin content range from of 3.83 to 17.08 mg/g, pectin content ranged from 5.17 to 29.44 per-cent. Cellulose ranged from 11.51 to 39.08 per-cent, whereas electrical conductivity ranged from 296.33 to 1012.15 ds/cm. Among trait associations, shattering score was negatively correlated with various pod biochemical traits including cellulose (-0.453), followed by starch (-0.424), lignin (-0.323) and pectin (-0.187) with no substantial relationship with EC. Lignin, pectin, cellulose and starch content in bean pod were significantly higher in the resistant genotypes compared to susceptible genotypes, whereas a reverse relationship was observed in case of EC content. The results in this study suggested that the chemical analysis of dry pod shells may provide useful information on breeding and selection of the resistant cultivars in common beans.

Keywords- Common bean, pod shattering, domestication syndrome, lignin, pectin

Introduction

Common bean is one of the most popular pulse crops known as “Poor man’s Meat” or “Grain of Hope”. With about 24 million tonnes produced globally with yields of about 824 kg/ha. Sub-Saharan Africa and Latin America account for about half of the common bean production followed by South and South-East Asia (35per cent). Global exports of common bean stand at 4.23 million tons (31per cent), only next to peas among pulses (1). Common bean is also regarded as a “nearly perfect food” as it contains a balanced mixture of different nutrients that promote better health and fight certain diseases. It has a rich nutritional value with about 22.8 per-cent proteins, low fat (~ 2%) and adequate dietary fibres (~20 per-cent). Compared to cereals, it has very low glycaemic index (24per cent) and is also rich in nutrients such as Iron (~ 9 mg/100g). India ranks second after Brazil in common bean production. In India, it is grown over an area of 1 M ha with a production of 0.4 MT and productivity of 400 kg ha⁻¹ (2). In Western Himalayas, the common bean is largely cultivated in Chamba, Kinnaur, Rajouri, Baderwah, Kishtwar, Uri, Kupwara, Shopian etc. There is great variation in farmer’s preference for the growth habit, and seed shape, colour, and size. In Ropa Valley of Kinnaur, red capsule types are more preferred, while as small-seeded red types are preferred in Salooni, Teesa, Kishtwar and Baderwah and upper tracts of Uttarkashi and Chamoli, yellow types are liked in Spiloo and Moorang areas, small white seeded are preferred in Kalpa and Rogi. In Chauhar valley in HP and Chadar and Dharam valley in Uttarakhand, mottled types are preferred (3).

In Jammu and Kashmir, Western Himalayan state of India, common bean (*Phaseolus vulgaris* L.) is locally known as *rajmash* and is the most important summer season pulse crop of hilly areas of Jammu and the entire Kashmir valley. The crop is mainly consumed as dry (mature) beans, shell beans (physiologically mature seeds) as well as green pods. It is an indispensable component of the diets as well as the farming system and enjoys a niche status among the pulse crops on account of being a cheap source of protein, minerals, and nutraceuticals. It is also a substantial contributor to the income of the subsistence farmers as it fetches better prices than cereals. The lack of high-yielding varieties that could fit in intensive farming systems has resulted in a fast dwindling of area under common bean. Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir (SKUAST-K) has released five *rajmash* varieties (Shalimar *Rajmash*-1, Shalimar *Rajmash*-2, Shalimar *Rajmash*-3, Shalimar *Rajmash*-4, and Shalimar French Bean-1) which also has not been able to make an appreciable dent to increase the yields, that continue to remain less than 1000 kg/ha. The situation is further worsened by the looming threats of climate change implications that are becoming more than obvious now, in the form of increasing frequency of droughts, extreme weather events and crop failures. There is an urgent need to identify common bean varieties that combine productivity with resilience. This requires in-depth characterization of natural variation in available genetic diversity for productivity and water stress adaptive traits.

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Among various domestication related traits, pod shattering is one of the important domestication syndromes. The loss of seed shattering occurred independently in several crops and in different areas of the world during the domestication of many food crops, as this loss was crucial for the adaptation of the plants to the agro ecosystem, to provide ancient farmers with easier and more abundant harvests (4). Among the legume crops, indehiscent phenotypes emerged in soybean and common bean, which were domesticated in the old world and the new world respectively (5). However, fully indehiscent phenotype emerged in common beans only after domestication with the development of snap varieties that are used to produce green beans due to the absence of fibre strings along the pod valves. In other domesticated commercial classes (e.g., dry beans) shattering traits are only reduced from that observable in wild populations. Thus, deciphering the physical, biochemical, and genetic basis of pod shattering is important for evolutionary studies, particularly to unravel the mechanisms of parallel evolution (6), and because this will provide breeders with key information to manipulate this trait to reduce yield loss. The shattering system of legume crops is distinct from that of cereals. In legumes, dehiscence is after the “hygroscopic movement” of 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 (7). The ability to undergo this movement has often been attributed to specific patterns of lignification of the pod-valve tissues. Among legumes, the most relevant studies on pod dehiscence have been conducted in soybean.

In agriculture, shattering is the dispersal of a crop's seeds upon ripening. From an agricultural perspective, this is generally an undesirable process, and in the history of crop domestication, several important advances have involved a mutation that reduced shattering instead of the seeds being dispersed as soon as the pods ripe, with mutant plants retaining the seeds for longer, which made harvesting much more effective. In common beans, pre-harvest shattering loss is the major form of yield loss by shattering. There has been strong human selection against pod shattering in domesticated beans, but some bean classes such as dry beans have retained higher levels of pod shattering, leading to yield losses and a constrained harvest window (8). This issue is more severe in semi-arid environments where pods become brittle and fracture more easily. An undesirable spin-off of shattering is the emergence of volunteer weeds in the subsequent growing season, that can impede future crop rotations as well as implicate seed purity. There is less published information available in common bean in terms of shattering losses, but reported losses are to the tune of 35% in *Glycine max* (L.) Merrill (9), 20% in *Brassica napus* L., 40–60% in *Vicia sativa* L. (7) and 50% in canola. The natural propensity for seed dispersal is an undesired agronomic trait that leads to substantial yield losses and inefficient harvesting. The level of shattering is strongly influenced by environmental dryness (8). In arid climates, soybean yield losses may go up to 100% (9). Since the available climate change models predict an increase in aridity, it is expected that the shattering losses may be aggravated especially in arid areas. As such, plant breeders need to introgressing shattering resistance into commercial varieties to mitigate the imminent yield losses. This requires an in-depth knowledge of mechanistic, physiological, biochemical and the underlying genetic basis of pod-shattering resistance. Pod shattering also creates a metabolic bottleneck as it implicates an energy cost on plants and limits the seed size (10). In fact, 100-seed weight of shattering resistant types in adzuki bean *Vigna*

angularis (Wild.) and yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) was higher than the wild types (11). Since the degree of pod coiling of pod walls is strongly influenced by thickness of the wall fibre layer, the increased pod wall fibre thickness leads to yield penalties by promoting pod-shattering as well as competing with seeds for photosynthates. Pods play an important role in encapsulating the developing seeds and protecting them from pests and pathogens. In addition to this protective function, photosynthetically active pod wall contributes assimilates and nutrients to fuel seed growth. Signals originating from the pod may also act to coordinate grain filling and regulate the reallocation of reserves from damaged seeds to those that have retained viability. Pods can regulate seed growth and maturation, particularly in members of the *Brassicaceae* family, 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. (12). The clefts are formed before the separation of pod halves along the parenchyma of the dorsal and ventral sutures (13). The developing cleft in dry pods results from the decaying parenchymatous cells between the two halves of the bundle cap (14).

In terms of the existing state of knowledge about the biochemical basis of pod shattering, only few published records are available in common bean, even though enough experimental evidence is available in other legumes such as soybean and Medicago, that point towards a definite biochemical basis of pod shattering. Specific activity of two hydrolytic (cellulase and poly-galactouranase) and two oxidoreductase (peroxidase and polyphenol oxidase) enzymes in the shattering and non-shattering zones of pod shell of shattering-resistant and susceptible variety of soybean (15). The continuous increase of cellulase activity at the shattering zone of susceptible variety indicates the involvement and role of this enzyme in the pod-shattering process. The relationship between chemical components (neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose (HCe), cellulose (Ce), uronic acid and calcium) of pod shell and pod dehiscence in soybean, was investigated using 25 soybean cultivars (16 susceptible and 9 resistant) (16). The correlation of the contents of chemical components with pod dehiscence was examined by principal component analysis. The multiple regression analysis of the relationship between per cent PD and the content of chemical components also showed that pod dehiscence was best predicted by the-two chemical components, [HCe] and [Ce], suggesting that the chemical analysis of dry pod shell may provide useful information on breeding and selection of the shattering resistant cultivars.

The relative contents of biochemical components in pod wall and reported that the biochemical components in pod walls had closer correlations with pod shattering with hemicellulose being the decisive factor (17). The pod-shattering resistance is associated with aberrant lignin distribution in inner sclerenchyma (18). Recently, the overview of various physical and biochemical factors underlying pod shattering and discussed the role of biochemical and histological parameters as surrogates for pod-shattering response as they provide key inputs for selecting contrasting genotypes based on differential lignification, pectin, fibre, cellulose and total carbohydrate content as well as enzymes such as endo-polygalacturonase and β -glucanase and hormones (19). In common bean, no comprehensive study has been done to study biochemical parameters underlying pod-shattering resistance. In the present study we attempted to study the biochemical basis of

pod shattering resistance in common bean in forty contrasting bean genotypes selected based on shattering response under random impact assessment.

MATERIALS & METHODS

Field experiment

Site of the experiment: The experiment was laid in 2021 at the research fields of the 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 soil of the experimental site is a typical incept sol 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/kg. All the accessions were grown as single rows of four-meter length, with spacing of 15 cm x 40 cm, in an augmented block design with four checks. The mean minimum and maximum temperatures (May-September) were 10.63 °C and 22.48 °C, with the lowest (16.43 °C) and highest (25.61 °C) maximum recorded in May and July respectively.

Experimental material: The material for the present study comprised of a core set of 254 lines including four checks (two state-released checks viz., Shalimar Rajmash-1, Shalimar French Bean-1 and two national released varieties viz., 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 colour classes and seed sizes and shapes. All the accessions were grown as single replicates in an augmented block design except the checks that were replicated in each block. Based on the shattering response using a random impact assessment forty contrasting lines (20 resistant and 20 susceptible) were selected for biochemical analysis.

Crop management: The management practices were uniform and homogeneous and comprised of seed treatment with the fungicide and the insecticide @ 2ml/kg seed, application of the pre-emergent herbicide Pendimethalin at a dose of 1.25l/ha as well as timely manual weeding, the 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 x 10 cm) where they equilibrated to constant moisture content for 10 days at room temperature.

Manual screening for pod shattering using Random Impact Method

Field based phenotypic evaluation of pod shattering requires fully grown plants, and it is a time-consuming and labour-intensive procedure (20). Moreover, the fluctuations in weather parameters at the time of pod maturation causes bias in the results. Screening of pod shattering was done in the laboratory's as per the method was suggested with modifications using an in house designed Random Impact Apparatus (RIA) comprising of a 20 cm diameter cylinder with six steel balls of 12 mm diameter (21). The pods harvested at maturity and equilibrated for moisture were oven dried at 80 degree for 2 days and 10 sampled pods were put in RIA and manually shaken for 10 seconds using a stopwatch (Figure 1). Each treatment was done in triplicate. The per-centage of pods shattered was recorded as

an estimate of pod shattering resistance. Data were recorded both before and after shaking the apparatus. The types of shattering response were done on scale of 1- 10as depicted in Figure 2.

Pod Biochemical Traits

Carbohydrate content: Carbohydrate content was estimated by using the Anthrone method (22). The main ingredient of anthrone is anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green colour in dilute and a blue colour in concentrated solutions, which is determined calorimetrically. The blue-green solution shows an absorption maximum at 620 nm. Briefly, 0.5 g sample was homogenized in hot 80 per-cent ethanol, centrifuged and residue was retained. The residue was washed repeatedly with hot 80per-cent ethanol till the washing did not give colour with anthrone reagent, followed by drying of the residue over a water bath. To the residue 5 ml of water and 6.5ml of 52 per-cent perchloric acid was added. The process was repeated using fresh perchloric acid. The supernatant was pooled and made up to 100ml. About 0.2ml of the supernatant was pipetted out and made up to 1ml with water, followed by the addition of 4ml of anthrone reagent. The mixture was heated for eight minutes in a boiling water bath, followed by rapid cooling and the intensity of green to dark green colour was read at 620nm. Standard was made from a 200µg glucose per mL distilled water stock solution using a range of concentrations. All the chemicals were procured from HI media.

Grain lignin content (AcBr method): Lignin content (mg/g) was estimated by the acetyl bromide (AcBr) (23). Protein free sample (20 mg) derived by sample preparation in phosphate buffer (pH 7.0) was placed into a screw-cap centrifuge tube containing 0.5 ml of 25 per-cent AcBr (v/v in glacial acetic acid) and incubated at 70°C for 30 min. After complete digestion, the sample was quickly cooled in an ice bath, and then mixed with 0.9 ml of 2 M Sodium hydroxide, 0.1 ml each of 5 M hydroxylamine-hydrochloric acid, and glacial acetic acid sufficient for complete solubilization of the lignin extract. After centrifugation (1,400xg, 5 min), the absorbance of the supernatant was measured at 280 nm. All the chemicals were procured from Himedia.

Grain pectin content: Pectin content (mg/g) was estimated by gravimetric method (24). A powdered sample weighing 0.5gram was put 0.01N hydrochloric acid and boiled for 30 minutes followed by filtration. The process was repeated using 0.05N and 0.3N hydrochloric acid in the same way total filtrate was collected. Two ml of the filtrate was pipetted out to which 5 ml distilled water and 1 ml sodium hydroxide solution was added. The solution was left for overnight. Next day, 1ml acetic acid was added and the mixture kept for five minutes, followed by the addition of 0.5 ml calcium chloride and stirring for some time and boiling for 2 minutes. The solution was pipetted through Whatman's filter paper and kept it overnight and weighed. All the chemicals were procured from Himedia.

Grain cellulose (Gravimetric method): Cellulose (%) was estimated by Gravimetric method of (25). A powdered sample of 0.5 gram was taken to which acetic acid, water and nitric acid was added in the ratio of 8:2:1 and the mixture kept in water bath for 4 hrs at 90°, followed by centrifugation at 10000 rpm for 10 min. After discarding the supernatant, the pellet was

repeatedly washed twice with ethanol, followed by centrifugation at 10000 rpm for 10 min. after removing the supernatant, the pellet was dried and weighed and the percentage derived based on original sample weight. All the chemicals were procured from Himedia.

Pod membrane permeability (EC): Pod membrane stability was measured in terms of electrical conductivity by EC meter in Deci siemens/cm. Equal weight of pod biomass excluding seeds were kept in large falcon tubes for 16 hours in distilled water at room temperature.

Data analysis: Data for physical traits was taken from five randomly selected plants in each genotype. The pattern of variation was shown in terms of violin plots created through JASP Software developed by CIMMYT. Variability in the traits was assessed in terms of descriptive statistics comprising of mean, range, and coefficient of variation (CV) derived from STAR software developed by IRRI. Pearson's correlation coefficients were computed as per the formula to calculate correlation coefficient between any two traits (26). The significance of correlation coefficient was tested by the form:

$$t = \sqrt{r(n-2)^2 / (1-r)^2}$$

Where,

n = Number of treatments, and

r = Correlation coefficient.

Principal component analysis for pod physical and biochemical traits was done by XLSTAT. The criteria followed for selecting the principal components to be included in further analysis was based on Eigen values of principal components (27). It was taken that Eigen values above unity indicated that the evaluated principal component weight is reliable (28). Path analysis to assess the relationship between for pod physical and biochemical traits and principal components was done through JASP software developed by CIMMYT. Diversity analysis was performed based on the hierarchical similarity among genotypes using Ward's method and Euclidean distances. From the PCA values, a Euclidean distance matrix was established to obtain a relative dendrogram. The entries were clustered using Ward's minimum-variance method.

RESULTS & DISCUSSION

Evolution of common bean pod

The detailed account of evolutionary events in the development of the common bean pod from the leaf (19). The pod in common bean evolved from a single leaf, where the leaf folds to cover the seeds (29). 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 as the dorsal suture corresponds to the fused margins of a modified leaf. There are four main functional cell layers in a pod wall viz., (a) An exocarp comprising of epidermal cells with thickened walls, (b) A mesocarp comprising of parenchymal cells, (c) An endocarp comprising of sclerenchyma and (d) An inner epidermis layer (13). The exocarp is a single-celled epidermal layered, mesocarp is a multi-layered, whereas endocarp comprises of two distinct cell layers (30). The vascular bundles develop thick walls at the sutures and the resulting structure is called the bundle cap. In the dorsal suture, dehiscence zone spans across the entire pod wall but, in the ventral suture it terminates at the fibre cap cells at the border between the bundle cap and the mesocarp (27). Upon maturity, a cleft or wedge is formed in the bean pod along the parenchyma

of the dorsal and ventral sutures, where the pod begins to open (27). A non-lignified separation layer (SL) remains throughout pod development (28). Upon maturity, the two celled layer in the DZ remains as a non-lignified abscission layer (AL). These thin layers of parenchyma tissue help in releasing the mature seeds. A microscopic ultrastructure of ventral suture of common bean pod is given in Figure 3.

Variability for pod biochemical traits

There was substantial variability in 5 pod biochemical traits in the 40 contrasting genotypes of common bean differing in pod shattering response (Table 1), selected out of the initial set of 254 genotypes. indicating significant diversity of the material in respect of studied traits. Pod lignin content had a mean value of 8.57 mg/g with a range of 3.83 to 17.08 mg/g. Highest pod lignin content was recorded in case WB-216 (17.08mg/g), followed by WB-1441 (15.94mg/g), SFB-1 (15.6mg/g) and WB-1129 (7.86 mg/g) while as lowest value for pod lignin content was recorded in WB-1439 (3.83mg/g). All the genotypes with higher lignin content were those with significant amount of resistance to manual shattering (shattering score of 1-3), indicating a substantial role for pod lignin in shattering response (Figure 4 and 5). Lignin enhances the hydrophobicity and thus increase the hardness and physical strength of cell walls (7). Lignification of the dehiscence zone creates the required mechanical tension to break it. In wild bean accessions, there is comparatively strong sclerenchyma tissue especially in the ventral sheath of bean pods as compared to the domesticated cultivars (7). Lignin is an important chemical component that imparts structural integrity to cell walls, stiffness and strength by cross-linking with cellulose. In *Lotus corniculatus*, shattering is mainly due to changes in the orientation of the cells in the pod wall, as well as unequal swelling and shrinkage, and lower lignification of the mesocarp (31). Similarly, in *Vicia sativa*, the lignified fibre cap cells and cell structure of mesocarp likely play a major role in preventing the two halves of the pericarp from separating.

Pectin content of pods had a mean value of 17.32 per cent with a range of 5.17 per-cent to 29.44 per cent. The highest pod pectin content was recorded in case N-1 (29.44 per cent), followed by WB-20-208 (28.20 per cent) and French Yellow (27.53 per cent) while as lowest value for pod pectin content was recorded in SR-1 (5.17 per-cent). As with lignin, shattering resistant lines had invariably higher pectin as compared to susceptible lines. Pectin has a definite role in cell adhesion (9) and pectin degrading enzymes such as β -glucanase aid in abscission (29). Pectin also forms calcium pectate with calcium in the cells that imparts greater strength to the pods. Similarly, endo-polygalacturonase aids in shattering by degrading pectin through hydrolysis of α -1,4-glycosidic bonds, which facilitates the breakdown of the middle lamella (30). Pectin is an important component of cell skeleton that maintains cell adhesion especially the homogalacturonan-rich pectin which is found in middle lamella between adjacent cells (31). In Fabaceae family, pod dehiscence is initiated by the weakening of the dorsal and ventral dehiscence zones, triggered by cell wall modifying enzyme EPG (endo-polygalacturonase), which is closely related to the pectin hydrolases that has been implicated in silique dehiscence processes in *Arabidopsis* and *Brassica* upon pod maturity and senescence.

Cellulose had a mean value of 23.15 per cent with a range of 11.51 per cent to 39.08 per cent. Highest cellulose was recorded in case WB-1518 (39.08 per cent), followed by WB-1189 (36.12 per cent) and WB-642 (34.32 per cent) while as lowest value for cellulose was recorded in PBG-716 (11.51 per cent). In addition

to lignin and pectin, cellulose alone as well as in combination with hemicellulose provides strength and structure integrity to cell wall that helps in increasing shattering resistance.

EC had a mean value of 605.57 ds/cm-with a range of 296.33ds/m to 1012.15ds/cm. The highest EC was recorded in case WB-1310 (1012.00 ds/cm), followed by G-1 (996.00 ds/cm) and G-3 (876.00 ds/m) while as lowest value for EC was recorded in WB-1439 (515.00 ds/cm). Pod carbohydrate content had a mean value of 8.57 per cent with a range of 13.36 per-cent to 48.60 per cent. The highest pod carbohydrate content was recorded in case WB-1528 (48.60 per cent), followed by WB-1189 (48.40per cent) and WB-642(48.20 per cent) while as lowest value for pod carbohydrate content was recorded in SR-3 (13.36per cent). In case of cowpea yard long bean cellulose and hemicellulose have been found highly correlated with shattering resistance on account of the formation of secondary thick wall layers of fibre cap cells. The range of trait dispersion as depicted by range and CV (%) value showed that highest CV value was observed in case of pectin (36.71per-cent) followed by lignin (35.33per-cent), EC (30.92per cent), starch (29.83per cent) and lowest CV was observed in cellulose (28.45per cent).

Trait association of pod shattering with biochemical traits

The Pearson correlation analysis of 5 pod biochemical traits with shattering score is presented in heat map (Figure 6) The heat map showing correlation of pod biochemical traits indicated that shattering score was negatively correlated with various pod biochemical traits including cellulose (-0.453), followed by starch (-0.424), lignin (-0.323) and pectin (-0.187) with no substantial relationship with EC. Among other traits starch was positively correlated with cellulose (0.456), followed by lignin (0.306) and pectin (0.181). the significant correlation between these biochemical components of pod indicates towards joint action of these components in driving the shattering resistance. In fact, lignin, pectin, and cellulose alone as well as in combination with hemicellulose provides strength and structure integrity to cell wall that helps in increasing shattering resistance (32). The linear relationship between pod wall biochemical compositions especially lignin, cellulose, and hemicellulose with shattering resistance in rapeseed mustard (18). As against the present study, did not find any significant variation in resistant and susceptible genotypes for cellulose content, even though there was variation in total carbon content of pods. Histological analysis has shown that shattering wild genotypes differ from non-shattering varieties in terms of the degree of lignification of the cells along the suture lines of the pod valves (33). Among the cultivated germplasm, differential lignification of the lignin-rich inner sclerenchyma of the pod walls also influences the level of shattering. The activity of two

hydrolytic (cellulase and polygalacturonase) exhibited continuous increase at the shattering zone of susceptible variety indicates the involvement and role of this enzyme in the pod-shattering process by breaking down cellulose and its linkages(32).The cellulose consists of glucose repeat units linked together in a manner that alternating molecules are rotated 180 degrees from each other. The orientation makes the cellulose stronger and provides greater strength to the pods especially the dehiscence zone. In *Medicago ruthenica*, polygalacturonase and cellulase activity analyses and RNA-sequencing (RNA-Seq), and RT-qPCR revealed a combination of two mechanisms viz., degradation of the middle lamella at the abscission layers and detachment of lignified cells on either side of the abscission, layer triggered by an increased polygalactouranase and cellulase activity in the pod ventral suture in the shatter-susceptible genotype. polygalacturonase and cellulase were highly expressed in the ventral sutures of the susceptible genotypes.

Comparative performance of genotypes for biochemical parameters.

The comparative analysis of pod biochemical traits in shattering resistant and susceptible genotypes are presented in Table 2 and Figure 7. The results indicate that the lignin, pectin, cellulose and starch content in bean pod were significantly higher in the resistant genotypes compared to susceptible genotypes, whereas reverse relationship was observed in case of EC content. The Lignin content in resistant genotypes was 9.69 mg/g as compared to 7.28 mg/g in susceptible genotypes. In case of pectin, the value was 19.29 per cent in resistant genotypes as compared to 16.37 per cent in susceptible genotypes. In case of cellulose the value was 26.00 per cent in resistant genotypes as compared to 20.71 per cent in susceptible genotypes. Similarly for starch content, the value was 35.73 per-cent in resistant genotypes as compared to 28.06 per cent in susceptible genotypes. The significant variation in total carbon content of pods in resistant and susceptible genotypes, even though there was no variation in for cellulose content of pods (21).

CONCLUSION

Common bean has retained the shattering trait. In view of the definite biochemical basis of shattering response, we can develop effective surrogates for improving shattering tolerance in common bean. No report is available in common bean regarding role of lignin, pectin, and other pod constituents in relation to pod shattering. The results of the present study suggested that the biochemical analysis of pod shell may provide useful information on the breeding and selection of the resistant cultivars.

Table 1: Descriptive statistics for biochemical traits related to pod shattering

Trait	Min.	Max.	Mean +SE	CVper-cent
Lignin	3.83	17.08	8.57 +0.484	35.33
Pectin	5.17	29.44	17.32 +1.017	36.71
Cellulose	11.51	39.08	23.15 + 1.052	28.45
Starch	13.36	48.60	31.05 +1.483	29.83
EC	296.33	1012.15	605.57 + 29.99	30.92

Table 2: Comparative biochemical profile of shattering resistant and susceptible genotypes

Shattering response	Lignin	Pectin	Cellulose	Starch
Resistant	9.693	19.263	26.00	35.734
Susceptible	7.281	16.375	20.708	28.063



Figure1: Protocol for random impact assessment of manual pod shattering in beans

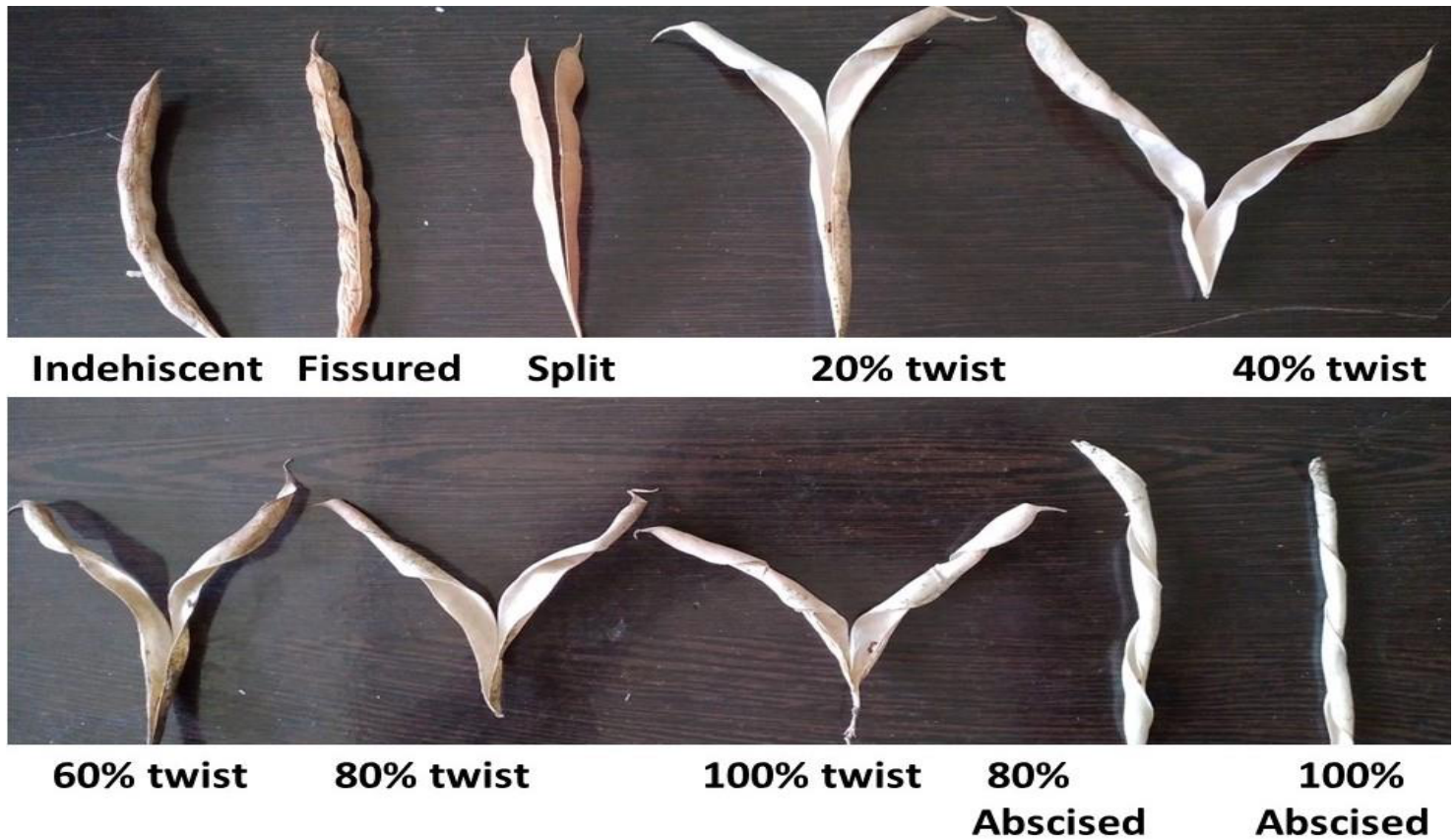


Figure2 : Types of shattering response in beans

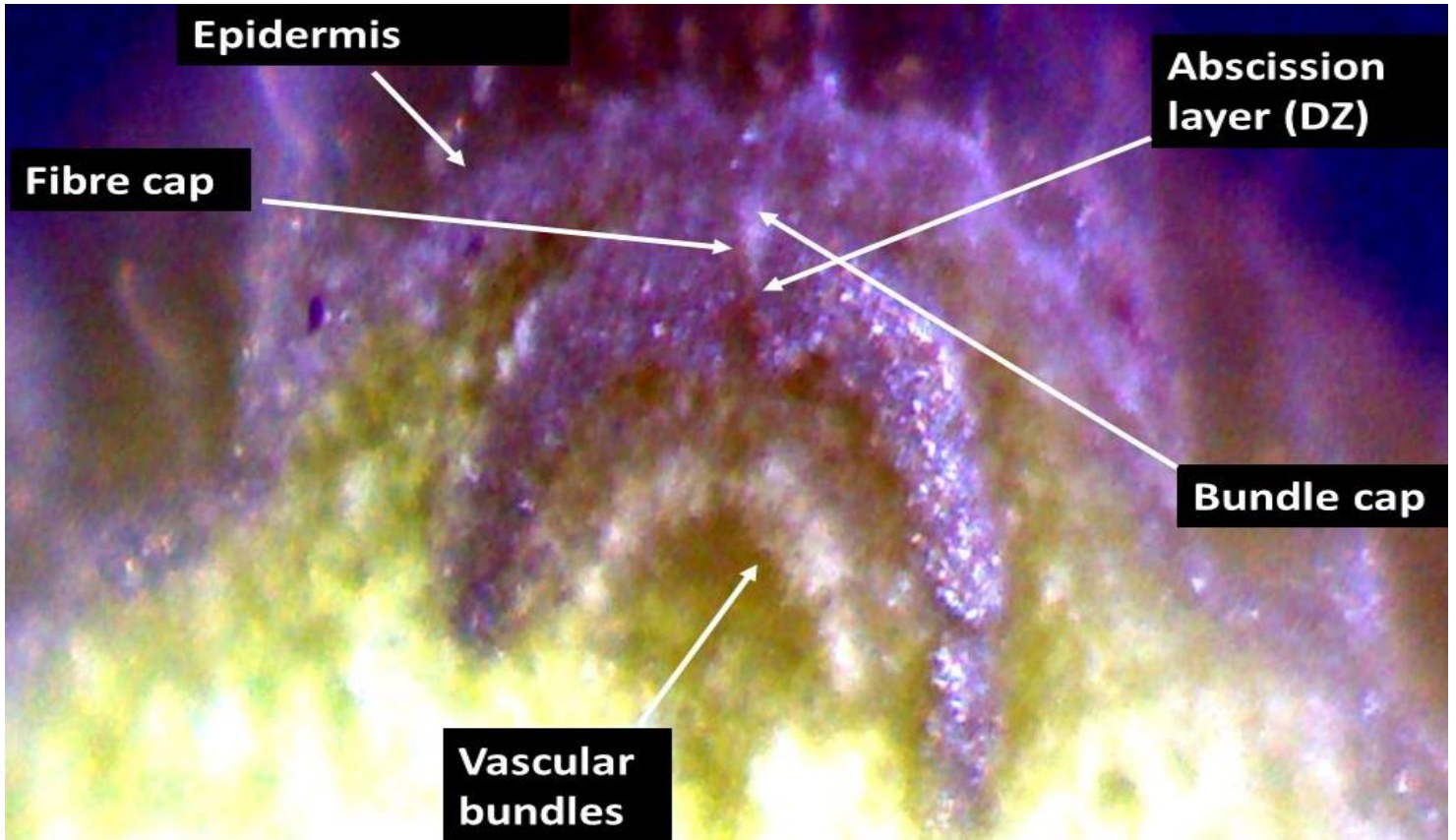


Figure3: Lignin deposition in pod wall and bundle cap region with dehiscence zone (Toluidine staining100x)



Figure 4: Differential response of a resistant (left) and susceptible (right) genotypes to manual shattering



Figure 5a : Response of a resistant genotype to RIA based screening

Figure 5b : Response of a susceptible genotype to RIA based screening

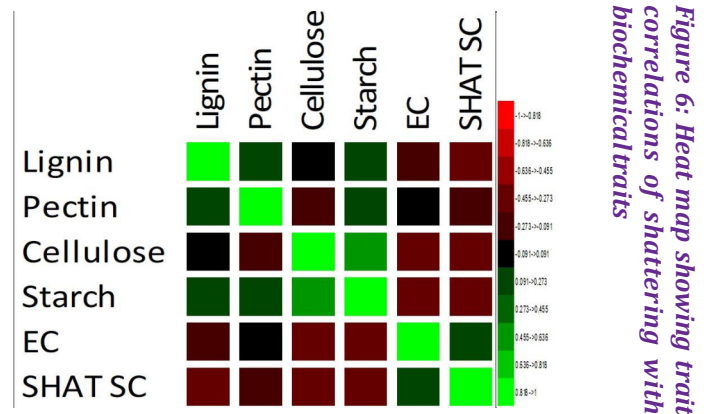


Figure 6: Heat map showing trait correlations of shattering with biochemical traits

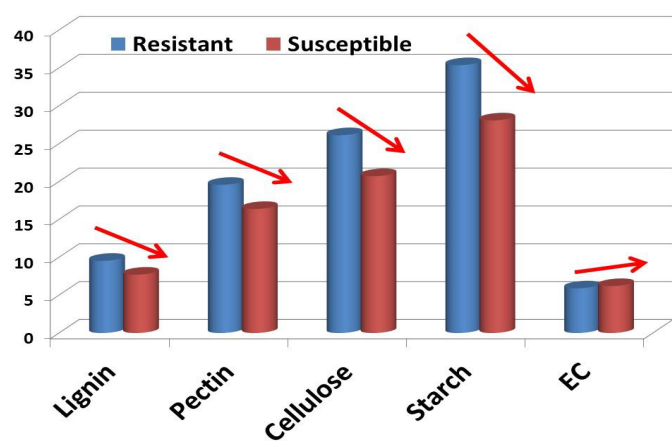


Figure7: Comparative analysis of pod biochemical traits in shattering resistant and susceptible genotypes

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