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Improvement in pod shattering trait: Evolutionary significance in domesticated crops

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Abstract
Indehiscent or non-shattering trait is one of the evolutionary significant event of crop domestication. In wild taxa, dehiscence is vital for the propagation of progeny and their adaptation under diverse growth conditions. Dispersing seeds from the maternal parent is essential, as greater distances generally amplify offspring success due to the availability of less competitive environments. In crop plants however, indehiscence is an ideal trait, because dehiscent fruits make harvesting complicated and often lead to significant production losses. Therefore, shattering was likely to be one of the first character robustly selected against by early agriculturalists. Although a vital trait, not all seed crops have wholly indehiscent fruits. In order to get better traits such as disease resistance and stress tolerance, breeders are often mandatory to utilize wild crop material, which are prone to shattering. Consequently, there is often some degree of shattering in cultivated material, predominantly in minor crops. Crop losses at harvest due to shattering can be extensive, especially in some conventional crops with a history of hand harvest, and transition to machine harvesting may further increase these losses. Statistics on crop losses from seeds shattered at harvest have not been systematically assembled, so their extent is not well known.

Keywords: Shattering, dehiscence, abscission, pod sutures, pod fibre

Introduction
Domestication is habitually described as a multi-step process. The most primitive farmers utilized the genetic variation present in the wild progenitors and selected individuals with favourable traits, improving the crop population. With selection and breeding, desirable characters in crop populations and crop varieties started to increase. After the preliminary stages of domestication, many crops experienced range expansions via human migrations and trade, and the limits to their present allocation are influenced by environmental factors. After domestication, deliberate breeding of crops further leads to variance of post-domestication traits, and improves yield and resilience in modern crops. The initial stage of domestication left its dent in current crop populations due to the truth that the early domestication efforts used a limited number of progenitors, which decreased the genetic diversity of the crop species. During domestication, the overall genetic diversity is abridged, and the effect is more pronounced in domestication-related genes as they are open to the elements to severe genetic bottlenecks due to strong selection.

Domestication-related reductions in pod shattering have occurred by manipulating the tension imposed by wall fibres and the potency of the sutures. These transitions have followed powerfully parallel trajectories in terms of both microscopic and macroscopic pod structure. This is an illustration of a Vavilovian homologous series (Vavilov, 1922) [19], in which a highly parallel range of phenotypes has been selected in a group of related but independently selected organisms. Unravelling the genetic and biochemical nature of these mutations is a quickly evolving field. Rau *et al*. (2019) [16] proposed that non-orthologous mechanisms were accountable for the loss of pod shattering in legumes, precisely reflecting the state of research at the time. Since then, an escalating body of evidence suggests that homologous genes can often rule variation in this trait between species (Di Vittori *et al*., 2020) [3], although several genes and mechanisms are accountable for this trait (Lenser and Theiben, 2013) [13]. In domesticated legumes, pod traits as a Vavilovian homologous series as follows.

Wild type pods
Domestication traits confer advantages in terms of no difficulty of harvest, survival in varying environments, and increased yield. These traits may reduce fitness in the wild but are preferred under human exploitation.

One such trait, pod shattering, is a necessary mechanism in wild legumes to spread their seeds and facilitate their propagation and reproduction. Greater dispersal distances created by shattering seeds are more likely to rest seeds in more distant micro-sites, away from pathogens and pests of the maternal parent and competition from siblings. From the agronomic perspective on the other hand, the natural tendency for seed dispersal is an undesired trait in crops as it leads to substantial yield losses and inefficient harvesting. Upon acquiring pod indehiscence, the continued existence of the crop depends on a symbiosis with a farmer, as the seeds must be disseminated by human labour. Consequently, natural seed dissemination was likely severely selected against by early farmers in the domestication process to assure efficient harvesting. The loss of shattering renders domesticated crops more reliant on human activity for propagation, and it further facilitates the fixation of other domestication characters, making it an significant milestone in the domestication process.

Histological fruit modifications related to seed shattering had been investigated in detail in *A. thaliana*, the mature silique is shaped by three dissimilar tissues: the valves, the replum, and the valve margins, which are situated between each valve and the replum (Figure 1). The valve margins correspond to the dehiscence zone, they consist of two further tissues: the separation layer and lignification layer. The lignification layer at the valve margin and an inside lignified valve layer (endocarp b) are essential for the creation of a mechanical tension in the dry silique before the detachment of the valves from the replum, that occur in the separation layer. In exacting, it has been shown that a lack of lignified and thickened secondary cell walls in the lignification layer of an *Arabidopsis* mutant silique result in the malfunction of seed shattering, diverse from the wild type, which shows fruit dehiscence. Moreover, it was shown that the short of a functional abscission layer (i.e., parting layer), along with ectopic lignification of the layer of cells that unite the valves and the replum in an *Arabidopsis* mutant, prevents silique dehiscence, as cell separation requires a specialized cell layer that is non-lignified and can undergo autolysis (Di Vittori 2019) [2].

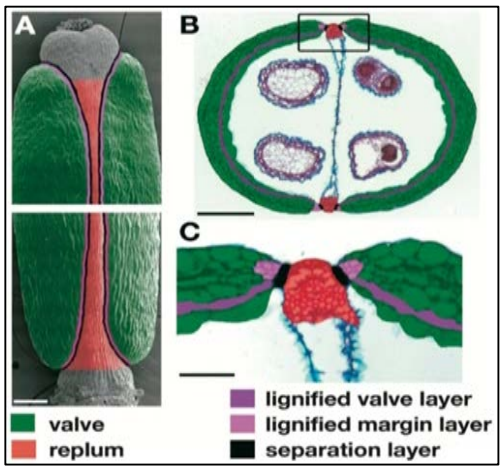

Fig 1: Representative scanning electron micrograph of mature wild-type fruit (stage 17) of A. thaliana. (A) Apex (top) and base (bottom) of fruit, with regions colored as indicated. (B) Transverse section with cell types colored corresponding to (A). Box: Valve margin region shown in (C). (C) Close up of valve margin region. Scale bars: 200 µm, (A, B); 50 µm; (C).

In few studies, the lignification patterns in the silique of *Cardamine hirsuta*, a relative of *Arabidopsis* that is considered by explosive seed shattering. There is strong asymmetric lignin deposition in the endocarp b cell walls of the fruit valves as accountable for the explosive seed shattering during silique opening (Figure 2). It was projected a model in which these "hinged cells" were required to store the mechanical tension that was required for the valve twisting. Indeed, when the dehiscence zone break, these hinges open, which allow the endocarp b to widen, thereby the different elasticity connecting the exo-carp and the endocarp b is accountable for the valve curling.

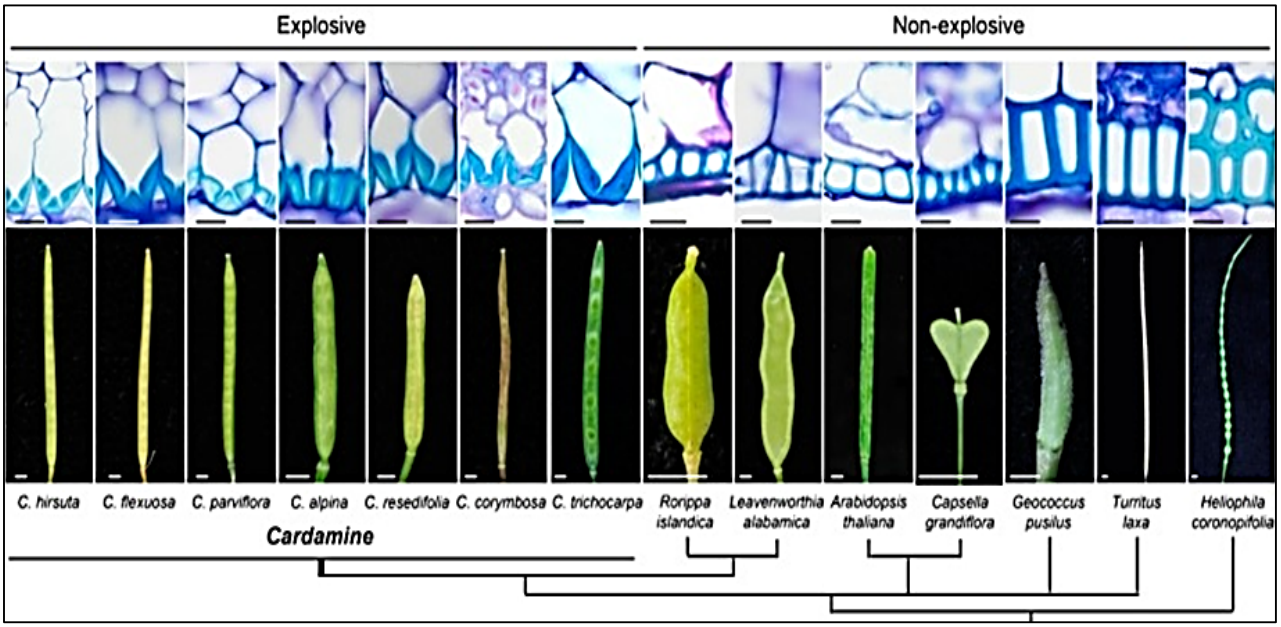

Fig 2: Representative patterns of secondary cell-wall lignin deposition in the endocarp b tissue for different species of the Brassicaceae family (as indicated) that are considered by explosive (Cardamine) and non-explosive silique shattering. Bottom: Mature wild-type fruit. Phylogenetic relationships connecting species are shown in the cladogram. Top: Light microscopy transverse valve section of fully grown fruit with cell walls stained with toluidine blue

Interestingly, assessment of the lignification pattern of the valves across several species of the *Brassicaceae* family, and asymmetric lignin deposition was noticed only in species of the *Cardamine* genus, which are the only ones in this family that are considered by explosive seed shattering.

In wild cereal species such as wheat and barley, seed shattering occurs when the spikelet separates from the rachis, which is the central axis of the spike. This phenotype is recognized as brittle-rachis, as a result of which the seeds drop to the ground (Figure 3). It was confirmed that, compared with the equivalent cell walls of the non brittle-rachis genotype, lower cell-wall thickness of both the primary and secondary cell walls of the separation layer (i.e., the connection where the spikelet break from the rachis) of wild barley result in disarticulation of the spikelets. This thus established that conservation of both the specific tissue (i.e., the abscission layer) and the secondary cell-wall thickening is necessary for the modulation of shattering.

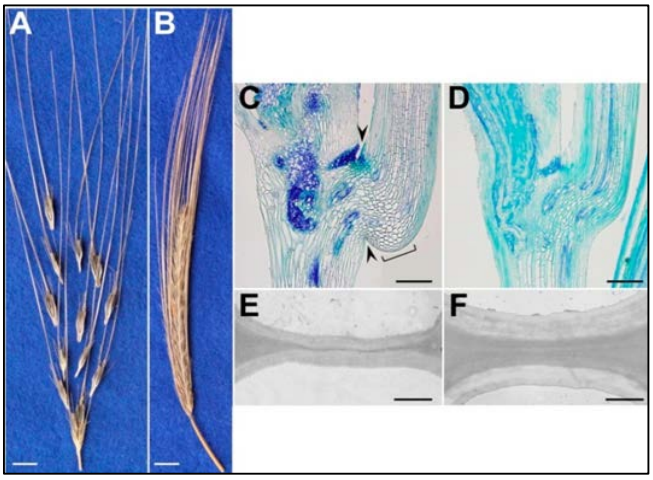

Fig 3: A: Mature spikes of wild barley accession (brittle) and B: induced non-brittle rachis mutant. (C, D) Longitudinal sections of connection between two rachis nodes at the anthesis stage, stained with toluidine blue O. Arrowheads: separation layer (or 'constriction groove'); square bracket: layer expanded cells. (E, F) Representative transmission electron microscopy indicating cell-wall thickness in separation layer of wild (E) and shattering-resistant mutant (F) spikes prior disarticulation. Scale bars: 1 cm (A, B); 250 µm, (C, D); 1 µm, (E, F)

Shattering occurs in cereals also with diverse mechanisms, that depend on the inflorescence architecture. In rice, which produce a panicle, the grain disarticulates near the pedicel, which is the last consequence that bears the flower on the inflorescence; in this species, the correct development of a particular abscission cell layer at the junction between the pedicel and the flower is required for grain scattering. In *Oryza nivara*, which is a wild rice species, has a uninterrupted abscission layer between the grain and the pedicel, while the domesticated *O. sativa* had an partial separation layer. However, a stronger grain attachment to the pedicel in *O. sativa* ssp. *japonica* accession, than in the *indica* cultivar, as, in the former, the abscission layer exhibited a higher degree of discontinuity. It is reported that *indica* cultivars show a comparatively high degree of seed shattering, while this trait was vanished in several *japonica* varieties. Human selection favored mutations that declined seed shattering in rice, even if the abscission layer is still incompletely developed also in the low shattering varieties. This process made it possible to decline yield losses due to the seed shattering, while a assured level of grain abscission is maintained to facilitate the threshing after the harvest.

In legumes such as the common bean and soybean, shattering occurs when the dry fruit open the length of the ventral suture. Although pods and spikes are entirely different fruit, their shattering resistance appears to result from a comparable and convergent mechanism. Indeed, improved secondary cell-wall thickening in the fiber cap cells of the ventral suture in domesticated soybean (*Glycine max*), compared with less-thickened cells of wild progenitor (*Glycine soya*) (Figure 4), leads to whole indehiscent plants, where the pods do not open the length of the ventral suture. Further, an internal lignified valve layer has been positively correlated with the shattering level in wild soybean, which recommended a parallelism with the lignified endocarp b of *Arabidopsis* that contributes to the modulation of shattering.

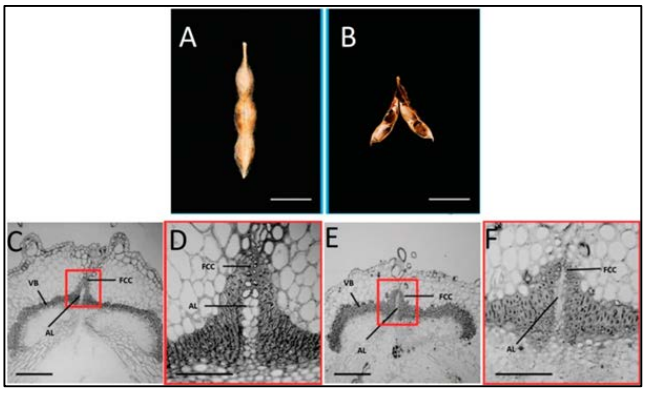

Fig 4: A: Mature pods of domesticated soybean (G. max) and B: wild soybean (G. soja). C–F: Cross-sections (~500 nm) of ventral sutures of domesticated and E, F: wild soybean pods. C, E: Boxes: Magnified regions shown in D, F. Details show fiber cap cells (FCC) at junction connecting two vascular bundle (VB) valves, with adjoining abscission layer (AL). Scale bars: 1 cm, in A, B; 200 mm, in C, E; 80 mm in D, F.

Improved fibre content in pod sutures and higher lignin content in pods are associated with the occurrence and mode of shattering in common bean (i.e., number of twisted pods/plant). Indeed, a elevated percentage of fibre cells (i.e., lignified and heavily thickened cells) in the ventral and the dorsal sheets of pods of stringy variety Wagenaar (i.e., high shattering type), when compared with the stringless pods of Fijne tros snap bean (i.e., indehiscent fruit), where there was a majority of wood cells across the sheats (i.e., lignified but not thickened cells) (Figure 5).

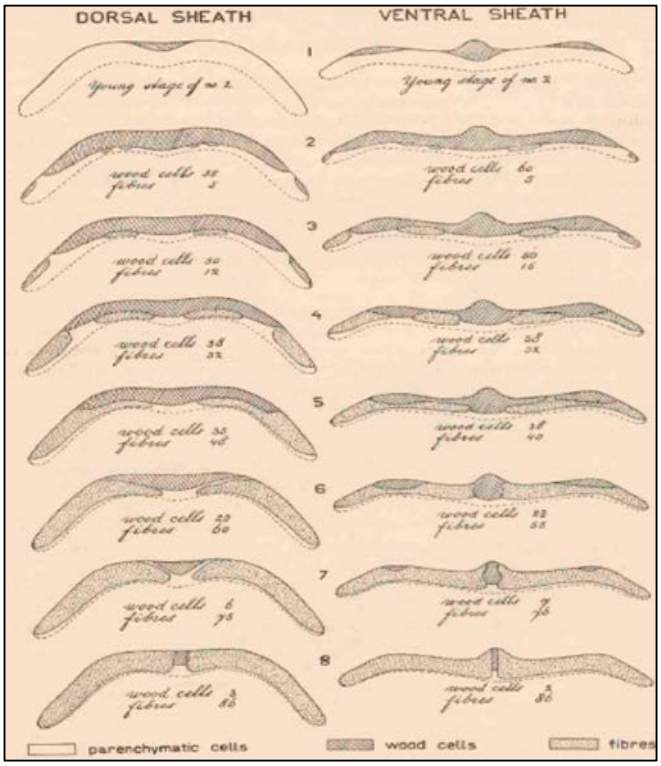

Fig 5: Pod fiber content in stringy and string less common bean varieties. Dorsal (left) and ventral (right) sheets of pods of stringy type Wagenaar (8 in Figure), string less type Fijne tros (2, 3 in Figure), intermediate F1 plants obtained after cross between Wagenaar and Fijine tros (4, 5, 6 in Figure), and young pods of the variety Fjine tros (1 in the Figure). Figure shows the distribution of parenchymatic (i.e., non lignified), wood (i.e., lignified but not thickened), fiber (i.e., lignified, heavily thickened) cells.

Interestingly, it was noticed positive correlation between the shattering level (i.e., number of shattered pods per plant) and valve weight, while the shattering level was negatively correlated with 100-seed weight and with quite a lot of descriptors of pod shape (i.e., pod perimeter, area, maximum width, maximum height, curved weight). They recommended an "energy cost" for the high-shattering plants due to the need for enhanced synthesis of molecules such as lignin and other fibres, result in plants with heavier pods, smaller seeds. However, the better fibre content might constantly create tension during fruit development, which would lead to the creation of curved and smaller pods in the shattering lines, compared to straighter pods of the non-shattering genotypes. Although the same data can be explained as arising through pleiotropic effects or linkage drag, pod shattering can be considered as a syndrome at the pod level.

The lignin content and tissues, there is lignification are vital factors for shattering, the length of with geometrical lignin deposition in cell walls and the environmental conditions. Indeed, few species such as *C. hirsuta* shatter because of high cell turgor of the silique, while other species such as legumes and *Arabidopsis* shatter after the fruit have entirely dried; thus, the drier the environment, the better their shattering susceptibility (Di Vittori 2019) [2].

Reduced twisting force of pod wall (disrupted fiber orientation, biochemistry, etc

Compared with their wild-growing progenitors, cultivated plants frequently show marked phenotypic differences although they belong to the same biological species. These differences, collectively confirmed the domestication syndrome, result from selection during several thousands of years for adaptation to the cultivated environments. Differences occur in characters such as seed dormancy, seed dispersal mechanisms. Investigations on genetic control of the domestication syndrome have generally focused on individual characters. Recently, a more comprehensive analysis has been made achievable by the availability of molecular linkage maps. The two most significant attributes of the domestication syndrome in common bean are the loss of seed dispersal ability and seed dormancy because they are essential for adaptation to a cultivated environment. The former is conditioned by the existence of fibres in pods, both in their sutures ("string") and their walls. Loss of these fibres leads to indehiscence of the pods and lack of seed scattering at maturity. Cultivated beans have, thus, effectively come to depend upon human intervention for their sustained survival. Cultivated beans also exhibit a more compact growth habit compared with their wild progenitor. Linkage data show that linkage group D1, and to a lesser extent linkage groups D2 and D7, had an effect on the domestication syndrome that was excessively large when considering their genetic length. Out of 16 quantitative characters, four on linkage group D2 (principally seed dispersal and dormancy) (Koinange *et al*, 1996) [12].

The genetic differences between mungbean and its presumed wild ancestor were analyzed for domestication associated characters by QTL mapping. In total genes for 38 domestication related characters were identified out of which Pod dehiscence is the major trait. Pod dehiscence decreases the number of seeds harvested. The number of twists down the length of the shattered pod (PDT) and the percentage of shattered pods one week (PDR1W), two weeks (PDR2W) and four weeks (PDR4W) after harvesting were used as the indices of pod dehiscence. As expected, the alleles from the cultivated parent reduced PDT. For PDR1W, PDR2W and

PDR4W, there were considerable differences in the percentage of shattered pods between the parents (Isemura *et al*., 2012) [10].

Pod dehiscence (shattering) is necessary for the propagation of wild plant species bearing seeds in pods but is a major reason of yield loss in legume and crucifer crops. Eventhough natural genetic variation in pod dehiscence has been, and will be, useful for plant breeding, little is known about the molecular genetic basis of shattering resistance in crops. A dirigent-like protein, shattering-resistant genotype, *pdh1*, was imperfect, having a premature stop codon. The functional gene, *Pdh1*, was highly articulated in the lignin-rich inner sclerenchyma of pod walls, especially at the stage of beginning in lignin deposition. Comparisons of near-isogenic lines indicated that *Pdh1* promotes pod dehiscence by rising the torsion of dried pod walls, which serves as a driving force for pod dehiscence under low humidity. Furthermore, the orthologs of *pdh1*, or genes with the same role, will perhaps be useful for crop improvement (Funatsuki *et al*., 2014) [6].

Common bean (*Phaseolus vulgaris* L.), most significant legume crop, developed a methodological pipeline that comprises a thorough characterization under field conditions, including also the chemical composition and histological analysis of pod valves. The pipeline was improved based on assumption that the shattering trait itself can be treated in principle as a "syndrome" (i.e., a set of correlated different traits) at pod level. Characterized a population of 267 intro-gression lines with the objectives: (1) to dissect shattering character into its "components," of *level* (percentage of shattering pods per plant) and *mode* (percentage of pods with twisting or non-twisting valves); (2) to test whether shattering is associated to chemical composition and/or the histological traits of the pod valves; and (3) to test associations among shattering and other plant traits. Results revealed the high shattering levels can be obtained in the different modes; shattering resistance is the mainly a qualitative character; and high shattering levels is correlated with the high carbon and lignin contents of the pod valves and with very specific histological characteristics of the ventral sheath and inner fibrous layer of pod wall. Shattering comes with a "cost," as it is connected with the low pod size, low seed weight per pod, high pod weight, and low seed to pod-valves ratio; indeed, it can be more exhaustively described as the syndrome at the pod level. The valve chemical composition (i.e., carbon and lignin content) can be used for a high trough-put phenotyping procedures for the shattering phenotyping (Murgia *et al*., 2017) [14].

Loss of pod shattering is one of the most essential domestication-related traits in legume crops. The non-shattering phenotypes have been achieved either by disturbed formation of abscission layer connecting the valves, or by loss of helical tension in sclerenchyma of endocarp, that split open the pods to disperse the seeds. During domestication process, azuki bean (*Vigna angularis*) and yard-long bean (*Vigna unguiculata* cv-gr. Sesquipedalis) have decreased or lost the schlerenchyma and thus the shattering behaviour of seed pods. Here we performed fine-mapping with backcrossed populations and narrowed the candidate genomic region down to 4 kbp in azuki bean and 13 kbp in yard-long bean. Among the genes located in these regions, found MYB26 genes encoded truncated proteins in azuki bean, yard-long bean, and even cowpea. As such, MYB26 could be a target gene for improving shattering phenotype in other legumes, such as soybean (Takahashi *et al*., 2020) [18].

Pod dehiscence is a key character in legumes due to its relevance for seed dispersal as well as yield losses. In chickpea (Cicer arietinum L.), the identification of major and minor genes controlling pod dehiscence is most important when wild genotypes are used to introgress germplasm in cultivated ones. Characterized phenotypically a RIL population from an inter-specific cross and utilised a candidate gene approach to identify orthologous to dehiscence-related genes. The segregation pattern in the RIL population suggests that the character is under oligogenic control. Through genome mapping and sequencing, developed DNA markers and identified the PDH1 gene as an very important regulator of pod shattering in chickpea. Results may help for the exploitation of wild germplasm resources in chickpea breeding programs and shed light on the relationships between the molecular and phenotypic variations in this important legume species (Aguilar-Benitez 2020) [1].

Strengthening of the dehiscence zone

A search in field populations of *Lupinus angustifolius* L. and L. *digitalus* Forsk. yielded two morphologically and genetically distinct lines in each species with markedly decreased pod-shattering at maturity. In all four lines, reduced shattering was noticed due to a single recessive gene, the two genes of both species being non-allelic and probably unlinked. Double homozygotes were obtained, and proved to be fully non-shattering in each species. The anatomical changes consequential in reduced- or non-shattering are of at least two types. In one type of each species there is union of the normally divided strips of sclerenchyma in the pod seams, alike to that in the non-shattering Strain 3535A of *L. luteus*. In the others species there is a weakening of the schlerified inner layer (endocarp) of the pod walls, similar to that in *L. albus*, *L. mutabilis*, and many other cultivated legumes. It is suggested that at least two independent homologous series of genes control pod-shattering in the genus Lupinus (Gladstones 1967) [7].

In the legume crop soybean *Glycine max* (L.) Merr which provides vegetable oils and proteins for humans, the key cellular feature of the shattering-resistant character lies in excessively lignified fibre cap cells (FCC) with the abscission layer unchanged in the pod ventral suture. NAC (NAM, ATAF1/2 and CUC2) gene *SHATTERING1-5* (*SHAT1-5*) functionally activates secondary wall biosynthesis and initiates the significant thickening of FCC secondary walls by expression at 15-fold the level of the wild allele, which is attributed to functional interruption of the upstream repressor. Strong artificial selection of *SHAT1-5* has resulted a severe selective sweep across ~116 kb on chromosome 16. This locus and regulation mechanism applicable to legume crop improvement. It was noticed that the excessively lignified fibre cap cells (FCC) endowed the domesticated soybean with pod shattering-resistance phenotype and were promoted by a NAC gene *SHAT1-5* by expression at 15-fold the level of the wild allele via repressor disruption. This regulatory alter is correlative with strong artificial selection of *GmSHAT1-5* during soybean domestication with hitchhiking result on closely linked loci across ~116 kb in chromosome 16 of the soybean genome. This mechanism is different from the one underlying grain shattering resistance of domesticated cereals (Dong *et al*., 2014) [5].

In Common vetch (*Vicia sativa* L.), one of the very important annual forage legumes globally because of its multiple uses and high nutritional content. However, when it matures, the pod dehiscence can results in severe loss of seeds. In this research, utilised eight shatter-susceptible vetch accessions and 16 shatter-resistant vetch accessions, which were studied and selected from 541 accessions, to compare and analyze the contributing factors related to pod dehiscence. Found that the shatter-susceptible vetches all have abscission layers and that the shatter-resistant vetches all absence of abscission layers. External valve margin cells, which have not reported in other plants to date, were situated externally to the junction of the fruit valve where the valve margin present, with the abscission layers down in the ventral suture. It was found that shatter-resistant vetches have markedly thick external valve margin cell walls and obviously fewer pod wall torsion laps than shatter-susceptible vetches, and there was no perfect difference in the pod thickness to width ratio. It was confirmed, abscission layers, external valve margin cells, and pod wall torsion laps are the important factors affecting pod dehiscence. Thus, this research lays the foundation to study the mechanism of vetch pod dehiscence (Dong *et al*., 2017) [4].

Though crossing wild relatives to modern cultivars is a usual means to introduce alleles of non shattering, an alternative is *de novo* domesticating wild species that are already tolerant to various kinds of stresses. In *vigna stipulacea* Kuntze, which has fast growth, short vegetative stage, and broad resistance to pests and diseases. Developed an ethyl methanesulfonate–mutagenized population and obtained three mutants with reduced seed dormancy and one characterised by reduced pod shattering. Further, crossed one of the mutants of less seed dormancy to the wild type and confirmed that the phenotype was inherited in a Mendelian manner. *De novo* assembly of *V. stipulacea* genome, and the following re-sequencing of the F2 progenies successfully identified some mutants associated with non-shattering. To evaluate pod shattering in the mutant lines, calculated the rate of shattering of the harvested pods which were completely dried in the incubator. Whereas the shattering rate was 100% in the wild type, it was 0% in the *rps1* mutant. The *rps1* mutant also exhibited a reduced twisting of the seed pod. The number of twists/cm in the pods was 0.371 ± 0.018 in the *rps1* mutant, which was less than half of the wild type (0.866 ± 0.022). Interestingly, the *isi1*, one of the mutants of seed imbibitions, also exhibited slightly reduced shattering rate (73.99 ± 18.53%) and number of twists/cm (0.579 ± 0.093). Other mutants also slightly reduced in number of twists/cm, but their shattering rate was 100%. It was also observed cross-sections of seed pods and found the *rps1* mutant did not form abscission layer between the valves at all. The *rps1* mutant almost completely lost the pod shattering behaviour because of suppressed formation of the abscission layer between the valves. Therefore, the mutation in *rps1* might be in a gene involved in the SHAT1-5 pathway. The responsible gene for *rps1* phenotype might be useful for solving shattering problem in other legumes because *rps1* phenotype was severer than soybean SHAT1-5. On the other hand, however, severe disruption in development of abscission layer could increase labor to thresh. In addition, though not significant, repeatedly observed that the *rps1* mutant exhibited slightly increased seed imbibitions compared to the wild type. Such pleiotropy, unless it has other mutations involved in seed dormancy, might be because secondary wall thickening plays important roles in shattering behaviour in seed pod and water permeability in seed coat (Takahashi 2019) [17].

The absence of pod wall fiber

In a study conducted to assess the genetic control of the domestication syndrome in common bean (*Phasiolus vulgaris* L.). A recombinant inbred population resulting from a cross between a wild and a cultivated common bean was subjected to molecular linkage mapping and evaluation in short-day and long-day environments. The genetic control of this syndrome in common bean involves genes that can have a large effect (>25-30%) and account for a substantial part of the phenotypic variation observed (>40-50%). The distribution of domestication syndrome genes appears as concentrations in three genomic regions with a major effect on the syndrome and one of which greatly affects growth habit and phenology, the other seed dispersal and dormancy, and a determining adaptation to a cultivated environment. Whereas the influence of genetic background and environment on the expression of some traits will have to be further analysed, however, that domestication of common bean could have proceeded rapidly (provided genetic diversity and selection intensity were high) and that evolution can proceed through changes involving a few genes with large effect rather than through a gradual accumulation of changes coded by few changes with small effects. The information presented here should lead to marker assisted selection experiments of introgression of additional genetic diversity into the cultivated common bean genepool (Koinange *et al*, 1996) [12].

Hairy vetch, *Vicia villosa* (Roth), is cover crop that does not show a typical domestication syndrome. Pod dehiscence reduces seed yield and creates weed problems to the subsequent crops. Breeding efforts aim to decline pod dehiscence in hairy vetch. To characterize pod dehiscence in species, we quantified visual dehiscence and force required to produce dehiscence among 606 genotypes grown among seven environments of the United States. For identifying potential secondary selection traits, we correlated pod dehiscence with various morphological pod characteristics and field measurements. Genotypes of hairy vetch expressed wide variation in pod dehiscence, from completely indehiscent to completely dehiscent ratings. Mean force to dehiscence was also varied widely, from 0.279 to 8.97 N among genotypes. No morphological characters were consistently correlated with pod dehiscence among environments where plants were grown. Results indicated the visual ratings of dehiscence would efficiently screen against genotypes with high pod dehiscence early in the breeding process. Force to dehiscence may be the necessary to identify the indehiscent genotypes during advanced stages of selection (Kissing Kucek *et al*., 2020) [11].

Few published studies have been evaluated PD in the genus *Vicia*. It was documented 15% to 46% PD in one Argentinian landrace evaluated at one location over the two years, but there was no studies have evaluated PD of hairy vetch among diverse germplasm or growing conditions. In common vetch (*Vicia sativa* L.), PD was varied widely (3% to 96%) among diverse. Common vetch lines differing in PD were exhibited 22 differentially expressed unigenes.

In other members of Fabeae tribe, domestication has successfully eliminated PD (e.g. *Pisum* sp.) or reduced the PD to very low levels relative to wild types (e.g. *Lens* sp) PD was

controlled by one to three dominant loci in lentil (*Lens* sp) and one to two dominant loci in pea (*Pisum* sp.). PD has been more extensively studied in the Phaseoleae tribe of Fabaceae. In soybean (*Glycine max* L. Merr.), transcription factor *SHATI-5* and gene *Pod dehiscence 1* (*Pdh1*) meditate and control PD. In common bean (*Phaseolus vulgaris* L.), various QTL have been identified among bean races, most documented being the *Stringless* (*St*) gene in snap beans (Petr Smýkal *et al*., 2015) [15].

PD is influenced by environmental conditions, length of the pod drying, and handling methods postharvest. With varying maturity timings, diverse genotypes can be exposed to differing weather conditions during pod development. Consequently, genotype by environment interactions can cloud genetic effects. More controlled measurements of PD, such as oven drying of the pods to standardize moisture and/or applying force to a pod to induce dehiscence were more associated with genetic effects than measuring PD under the field conditions. Such methods, particularly measuring force needed to induce dehiscence, demand substantial phenotyping time and specialty equipment. Identification of the traits that are easier to measure and are highly correlated with PD could improve the breeding efficiency. Such secondary selection traits could accelerate improvement of the hairy vetch. Wide variation in the visual and force to dehiscence existed among the diverse genotypes. More importantly for selection, multiple lines exhibited indehiscence or very low levels of dehiscence.

This data*set al*so demonstrated the environmental influence on PD, which is well documented in other species. Growing environment contributed the substantial amounts of variance for visual dehiscence and force to dehiscence. Moreover, correlations between the metrics of PD, pod morphology, and flowering maturity significantly differed among the environments. To separate the genetic effects from environmental influences and interactions, PD studies should utilize the multiple environments. Secondary selection traits to speed phenotyping would need to consistently correlate among the diverse environments within a breeding program region of interest.

Spiraling was highly correlated with the PD and was a high-throughput measurement, requiring only 15 seconds per the sample. Visual dehiscence provided the higher resolution in PD than spiralling and was moderately time intensive, requiring 5 min to rate per line, at 50 pods evaluated per line. Force to dehiscence was most involved measurement, requiring the specialty equipment, a trained operator, and 18.5 min of evaluation time per line, with five pods evaluated per line. Although visual dehiscence and spiraling may be adequate to identify the strongly dehiscent lines, force to dehiscence may be the useful for identifying extreme lines most resistant to dehiscence. For initial screenings of dehiscence, spiraling could identify the genotypes which are most susceptible to PD at low cost. Visual dehiscence would be the useful in early and middle stages of selection to eliminate moderately dehiscent lines. Once mean visual dehiscence levels become low (< 1) in a breeding population, force to dehiscence measurements would likely be necessary to further advance gains in selection (Kissing Kucek *et al.*, 2020) [11].

Although pod morphology metrics were fast to measure (15 seconds per line), none were strongly related to the PD among environments. Pod corrugation was moderately correlated with all the measures of PD, and explained a large portion of variance for the visual dehiscence. Pith tissue was moderately correlated with the force to dehiscence and pod spiralling. However, pod corrugation and pith tissue did not commonly appear at three environments in the northern United States. Consequently, pod corrugation and pith tissue would not be useful PD secondary selection traits for the breeding programs including cold temperate climates.

Further study is needed to understand physiology of pith tissue in hairy vetch pods. The pith tissue created a foam-like structure that seemed to inhibit the compression force from breaking a pod, hence the trait's contribution to force to dehiscence. However, the pith tissue may not be the genetic resistance to PD, but rather a plant response to an environmental threat (e.g. a pathogen). To separate out environmental effects from true PD, trait of pith tissue could serve as a covariate when analyzing force to dehiscence.

The fracture structure of pod wall was moderately related to spiraling, and with visual dehiscence at some environments. The linear fracture morphology described in our paper likely relates to the alignment of the pod wall fibers at an angle to pod sutures, which can cause spiraling of the carpel. As the evaluation of the spiraling required equal time to measure as fracture, and spiraling was more correlated to other metrics of PD, we see little utility for a rating of fracture.

Some of the traits showed inconsistent correlation with PD metrics among environments, such as pod flexibility. Such traits would not be reliable for the secondary selection traits for PD. Pod moisture was not correlated with the visual dehiscence or force to dehiscence. Consequently, pods in our study had likely reached critical pod moisture required for PD. The weak correlation between spiraling and pod moisture could indicate that some samples were above critical pod moisture threshold for PD. Moisture contents in our evaluation (6.7% to 9.3%) were below critical pod moisture (10.1% to 10.4%) associated with PD in soybean. However, these moisture contents were above stable moisture found in common vetch (5%). Results of PD after various pod drying times, heat conditions, and pod moistures to identify the critical pod moisture in hairy vetch.

Flowering timing of the lines were not strongly correlated with any measures of PD. In other species, genotypes with the earlier flowering timing have exhibited more PD, as they were exposed for more time to heat and drying forces that can cause the rupture of the dehiscence zone. In our dataset, the stabilization of the pod moisture *via* drying may have reduced the influence of maturity timing on PD.

Selecting for the pod indehiscence may conflict with other field traits of interest in *Vicia villosa*. Lines with high spring vigor, a trait desired by the growers, also tended to have low PD, indicating potential to select for both desired traits. However, there was a trade-off between PD and the seed yield in some environments. Selection for the PD should closely monitor seed yield, to ensure the lines developed for low PD also produce adequate yield for seed growers. Kissing Kucek, 2020 [11].

Major reduction in suture fiber

Snap bean (*Phaseolus vulgaris* L.) breeding programs are tasked with developing cultivars that meet standards of the vegetable processing industry and ultimately that of the consumer, while matching or exceeding the field performance of existing cultivars. While traditional breeding methods have

had a long history of meeting these requirements, genetic marker technology, combined with the knowledge of important quantitative trait loci (QTL), can accelerate breeding efforts. In contrast to the dry bean, snap bean immature pods and seeds are consumed as a vegetable. Several pod traits are the important in snap bean including: reduced pod wall fibre, absence of pod suture strings, and thickened, succulent pod walls. In addition, snap bean pods are selected for the round pod cross section, and pods tend to be longer with cylindrical seed shape. Seed colour is an important trait in snap bean, especially those used for the processing, as processors prefer the white-seeded cultivars. RR6950, a small seeded brown indeterminate type IIIA dry bean accession, was crossed to Oregon State University (OSU) breeding line OSU5446, a type I Blue Lake four-sieve breeding line to produce RR138 $F_{4:6}$ recombinant inbred (RI) mapping population. The RR138 population was genotyped with BARC Bean 6K_3 Bead chip, and single nucleotide polymorphisms (SNPs) were used to assemble linkage map, and identify QTL for the pod traits. The map was populated with the average of one SNP per 1.4 cm, spanning 11 linkage groups. Overall, seed and flower colour genes *B* and *P* were located on Pv02 and Pv07, respectively. A QTL for string: pod length (PL) ratio was found on Pv02 controlling 32% of the total genetic variation. QTL for a suite of important processing traits including the pod wall fibre, pod height, pod width, and pod wall thickness were found clustering on Pv04 and controlled 21%, 26%, 18%, and 16% of genetic variation for each of these respective traits (Hagerty *et al*., 2016) [9].

In snap bean, pod wall fiber and pod suture fiber are the separate traits, and there has been some question as to whether they are under independent genetic control. For these parents two traits are independent because a QTL for pod suture strings was observed on Pv02 whereas a QTL for pod wall fiber appeared on Pv04. Paradoxically, two parents had similar pod wall fiber ratings. We would have expected that pod walls of OSU5446 would have had less fiber than walls of RR6950. Similarity in parents may have been the result of scale employed. It was a 3-point scale and may have been too coarse to account for the subtle differences in the parents. Another possibility has to do with high frequency of reversions to high fiber pods that are observed in snap bean. These reversions occur spontaneously at the rates of 0.5% to 2.25% (unpublished data), and it may have been that OSU5446 parent used for phenotyping pod wall fiber was such a revertant.

With exception of the pod suture strings, the QTLs are novel and have not been previously identified. PL showed positive phenotypic correlation with the pod width and pod height; however, PL was not genetically correlated because it was found on the Pv09, whereas the pod height and pod width were observed on Pv04. Therefore, if a larger or smaller sieve size bean is desired by the breeding program, this could be achieved independently of PL. While pod wall fiber was not genetically associated with the pod suture strings, it was positively correlated phenotypically and genotypically with the pod width and height (Lyle Wallace *et al.*, 2018) [8].

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