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## POD SHATTERING TRAIT AS A VAVILOVIAN HOMOLOGOUS SERIES IN DOMESTICATED CROPS

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### ABSTRACT

Indehiscent or non-shattering trait is one of the hallmarks of crop domestication. In wild taxa, dehiscence is crucial for the propagation of offspring and their adaptation under diverse growth conditions. Dispersing seeds from the maternal plant is important, as greater distances generally increase offspring success due to the availability of less competitive environments. In crop plants however, indehiscence is a preferred trait, because dehiscent fruits make harvesting difficult and often lead to significant production losses. Therefore, shattering was likely to be one of the first traits strongly selected against by early agriculturalists. Although a critical trait, not all seed crops have completely indehiscent fruits. In order to improve beneficial traits such as disease resistance and stress tolerance, breeders are often required to utilize wild crop material, which are prone to shattering. Consequently, there is often some degree of shattering in cultivated material, particularly in minor crops. Crop losses at harvest due to shattering can be substantial, especially in some traditional 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 thoroughly assembled, so their extent is not well known.

**KEY WORDS:** *Shattering, Dehiscence, Abcission, Pod sutures, Pod fibre*

## INTRODUCTION

Domestication is often described as a multi-step process. The earliest 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 traits in crop populations and crop varieties started to increase. After the initial stages of domestication, many crops experienced range expansions via human migrations and trade, and the limits to their present distribution are influenced by environmental factors. After domestication, deliberate breeding of crops further leads to divergence of post-domestication traits, and improves yield and resilience in modern crops. The initial stage of domestication left its imprint in current crop populations due to the fact 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 reduced, and the effect is more pronounced in domestication-related genes as they are exposed to severe genetic bottlenecks due to strong selection.

Domestication-related reductions in pod shattering have occurred by modifying the tension imposed by wall fibers and the strength of the sutures. These transitions have followed strongly parallel trajectories in terms of both microscopic and macroscopic pod structure. This is an example of a Vavilovian homologous series (Vavilov, 1922), in which a highly parallel range of phenotypes has been selected in a group of related but independently selected organisms. Unraveling the

genetic and biochemical nature of these mutations is a rapidly evolving field. Rau et al. (2019) proposed that non-orthologous mechanisms were responsible for the loss of pod shattering in legumes, accurately reflecting the state of research at the time. Since then, an increasing body of evidence suggests that homologous genes can often govern variation in this trait between species (Di Vittori et al., 2020), although several genes and mechanisms are responsible for this trait (Lenser and Theiben, 2013). Pod traits as a Vavilovian homologous series in domesticated legumes as follows;

### WILD TYPE PODS

Domestication traits confer advantages in terms of ease of harvest, survival in varying environments, and increased yield. These traits may decrease fitness in the wild but are preferred under human exploitation. One such trait, pod shattering, is an essential mechanism in wild legumes to spread their seeds and facilitate their propagation and reproduction. Greater dispersal distances generated by shattering seeds are more likely to place seeds in more distant micro-sites, away from pathogens and pests of the maternal plant and competition from siblings. From the agronomic perspective on the other hand, the natural propensity for seed dispersal is an undesired trait in crops as it leads to substantial yield losses and inefficient harvesting. Upon acquiring pod indehiscence, the survival of the crop depends on a symbiosis with a farmer, as the seeds must be dispersed by human labor. Consequently, natural seed dispersal was likely severely selected against by early farmers in the domestication process to assure efficient harvesting. The loss of shattering renders

domesticated crops more dependent on human activity for propagation, and it further facilitates the fixation of other domestication characters, making it an important milestone in the domestication process.

Histological fruit modifications related to seed shattering have been investigated in detail in *A. thaliana*, the mature silique is formed by three different tissues: the valves, the replum, and the valve margins, which are located between each valve and the replum (Figure 1). The valve margins correspond to the dehiscence zone, and they comprise two further tissues: the lignification layer and the separation layer. The lignification layer at the valve margin and an internal lignified valve layer (endocarp b) are required for the

creation of a mechanical tension in the dry silique before the detachment of the valves from the replum, that occurs in the separation layer. In particular, it has been shown that a lack of lignified and thickened secondary cell walls in the lignification layer of an *Arabidopsis* mutant silique results in the failure of seed shattering, different from the wild type, which shows fruit dehiscence. Moreover, it was shown that the lack of a functional abscission layer (i.e., separation layer), along with ectopic lignification of the layer of cells that connect 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).

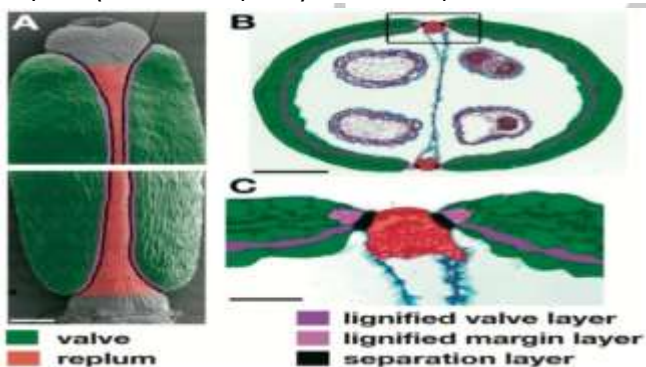


Figure 1. Representative scanning electron micrograph of mature wild-type fruit (stage 17) of *A. thaliana*. (A) Apex (top) and base (bottom) of fruit, with regions colored as indicated. (B) Transverse section with cell types colored corresponding to (A). Box: Valve margin region shown in (C). (C) Close 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 characterized by explosive seed shattering. There is strong asymmetric lignin deposition in the endocarp b cell walls of the fruit valves as responsible for the explosive seed shattering during silique opening (Figure 2). It was proposed

a model in which these “hinged cells” were required to store the mechanical tension that was needed for the valve twisting. Indeed, when the dehiscence zone breaks, these hinges open, which allows the endocarp b to widen, whereby the different elasticity between the exocarp and the endocarp b is responsible for the valve curling.

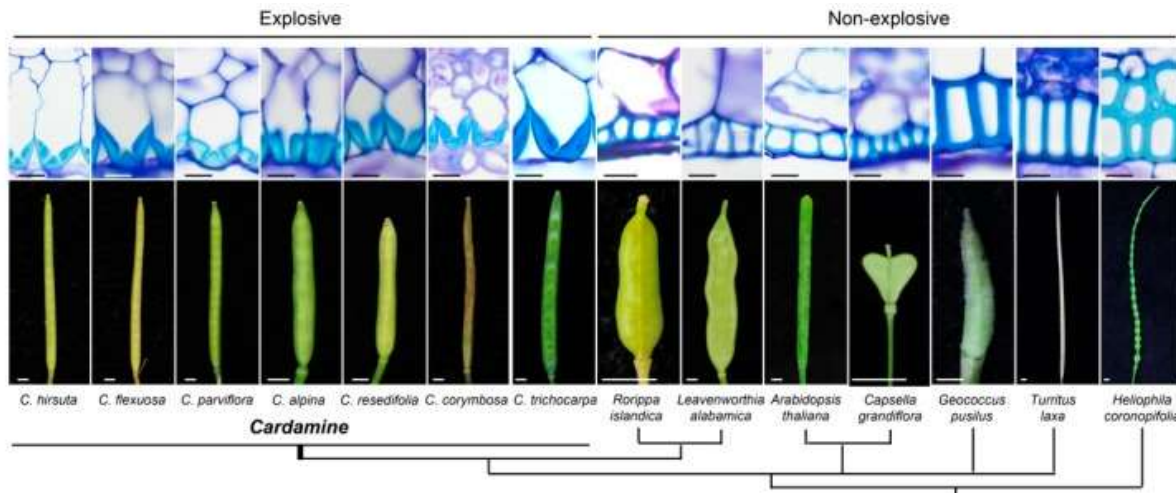


Figure 2. Representative patterns of secondary cell-wall lignin deposition in the endocarp tissue for various species of the Brassicaceae family (as indicated) that are characterized by explosive (Cardamine) and non-explosive silique shattering. **Top:** Light microscopy transverse valve sections of mature fruit with cell walls stained with toluidine blue *O*. **Bottom:** Mature wild-type fruit. Phylogenetic relationships between species are shown in the cladogram.

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

In wild cereal species such as wheat and barley, seed shattering occurs when the spikelet detaches from the rachis, which is the central axis of the spike. This phenotype is known as brittle-

rachis, as a result of which the seeds fall to the ground (Figure 3). It was demonstrated 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 junction where the spikelet breaks from the rachis) of wild barley results in disarticulation of the spikelets. This thus confirmed that conservation of both the specific tissue (i.e., the abscission layer) and the secondary cell-wall thickening is required for the modulation of shattering.

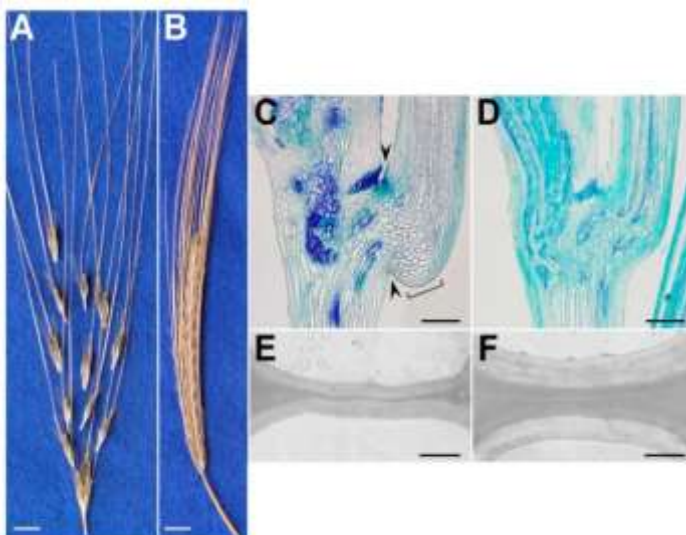


Figure 3. Representative mature spikes of wild barley accession OUH602 (A; brittle) and induced non-brittle rachis mutant M96-1 (B). (C,D) Representative longitudinal sections of junction between two rachis nodes at the anthesis stage, stained with toluidine blue *O*. Arrowheads: separation layer (or 'constriction groove'); square bracket: layer of expanded cells. (E,F) Representative transmission electron microscopy showing cell-wall thickness in separation layer of wild (E) and shattering-resistant mutant (F) spikes prior to disarticulation. Scale bars: 1 cm (A,B); 250 μm, (C,D); 1 μm, (E,F).



Shattering occurs in cereals also with different mechanisms, that depend on the inflorescence architecture. In rice, which produces a panicle, the grain disarticulates at the pedicel, which is the last ramification that bears the flower on the inflorescence; in this species, the correct development of a specialized abscission cell layer at the junction between the pedicel and the flower is required for grain dispersal. In *Oryza nivara*, which is a wild rice species, has a continuous abscission layer between the grain and the pedicel, while the domesticated *O. sativa* had an incomplete separation layer. Moreover, 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 showed a higher degree of discontinuity. It is reported that *indica* cultivars show a relatively high degree of seed shattering, while this trait was lost in several *japonica* varieties. Human selection favored mutations that reduced seed shattering in rice, even if the abscission layer is still partially developed also in the low shattering varieties. This process made it possible to

reduce yield losses due to the seed shattering, while a certain 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 along the ventral suture. Although pods and spikes are completely different fruit, their shattering resistance appears to result from a similar and convergent mechanism. Indeed, increased secondary cell-wall thickening in the fiber cap cells of the ventral suture in domesticated soybean (*Glycine max*), compared with the less-thickened cells of the wild progenitor (*Glycine soya*) (Figure 4), leads to complete indehiscent plants, where the pods do not open along the ventral suture. Moreover, an internal lignified valve layer has been positively correlated with the shattering level in wild soybean, which suggested a parallelism with the lignified endocarp of *Arabidopsis* that contributes to the modulation of shattering.

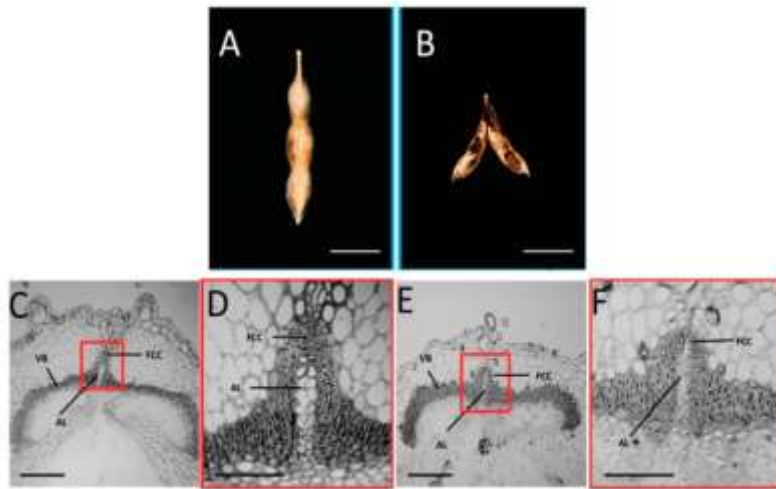


Figure 4. Representative mature pods of domesticated soybean (*G. max*) (A) and wild soybean (*G. soja*) (B). (C–F) Representative cross-sections (~500 nm) of ventral sutures of domesticated (C,D) and wild (E,F) soybean pods. (C,E) Boxes: Magnified regions shown in (D,F). Details show fiber cap cells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

Increased 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 per plant). Indeed, a high percentage of fibre cells (i.e., lignified and heavily thickened cells) in the ventral and the dorsal sheets of

Pods of the stringy variety Wagenaar (i.e., high shattering type), when compared with the stringless pods of the Fijne tros snap bean (i.e., indehiscent fruit), where there was a predominance of wood cells across the sheaths (i.e., lignified but not thickened cells) (Figure 5).

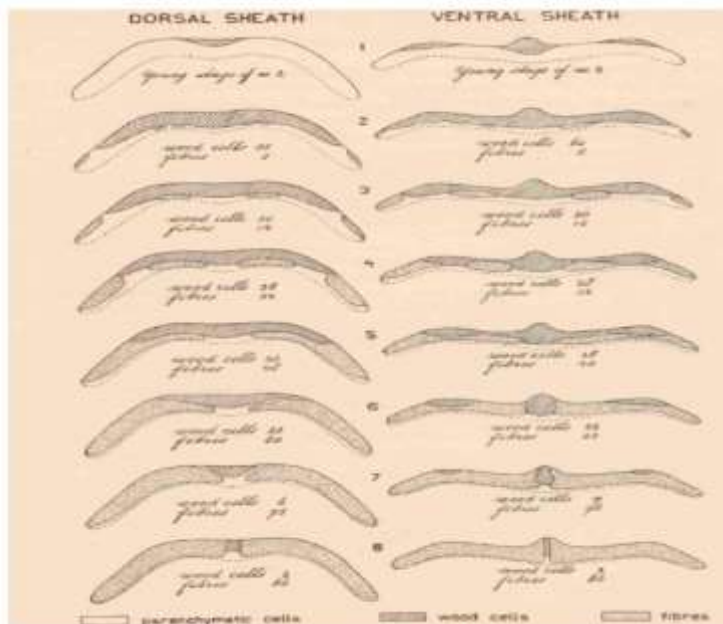


Figure 5. Illustration of the pod fiber content in stringless and stringy common bean varieties. Dorsal (left) and ventral (right) sheaths of pods of stringy type Wagenaar (8 in the Figure), stringless type Fijne tros (2 and 3 in the Figure), intermediate F1 plants obtained after the cross between Wagenaar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

Interestingly, it was observed positive correlation between the shattering level (i.e., number of shattered pods per plant) and the valve weight, while the shattering level was negatively correlated

with the 100-seed weight and with several descriptors of pod shape (i.e., pod perimeter, area, maximum width, maximum height, curved weight). They suggested an “energy cost” for the high-

shattering plants due to the need for increased synthesis of molecules such as lignin and other fibres, which result in plants with heavier pods and smaller seeds. Moreover, the increased fibre content might constantly create tension during fruit development, which would lead to the formation of curved and smaller pods in the shattering lines, compared to the 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 viewed as a syndrome at the pod level.

The lignin content and tissues where there is lignification are crucial factors for shattering, along with geometrical lignin deposition in the cell walls and the environmental conditions. Indeed, some 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 completely dried; thus, the drier the environment, the greater their shattering susceptibility (Di Vittori 2019).

#### **REDUCED TWISTING FORCE OF POD WALL (DISRUPTED FIBER ORIENTATION, BIOCHEMISTRY, ETC.**

Compared with their wild-growing progenitors, cultivated plants often show marked phenotypic differences although they belong to the same biological species. These differences, collectively called the domestication syndrome, result from selection during several thousands of years for adaptation to cultivated environments. Differences occur in traits such as seed dormancy, seed dispersal mechanisms. Investigations on the genetic control of the domestication

syndrome have generally focused on individual traits. Recently, however, a more comprehensive analysis has been made possible by the availability of molecular linkage maps. The two most important attributes of the domestication syndrome in common bean are the loss of seed dispersal ability and seed dormancy because they are crucial for adaptation to a cultivated environment. The former is conditioned by the presence of fibers in the pods, both in their sutures ("string") and their walls. Loss of these fibers leads to indehiscence of the pods and lack of seed dispersal at maturity. Cultivated beans have, thus, effectively come to depend upon human intervention for their continued survival. Cultivated beans also display 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 disproportionately large when considering their genetic length. Out of 16 quantitative traits, four on linkage group D2 (principally seed dispersal and dormancy) (Koinange et al,1996).

The genetic differences between mungbean and its presumed wild ancestor were analyzed for domestication related traits by QTL mapping. In total genes for 38 domestication related traits were identified out of which Pod dehiscence is the major trait. Pod dehiscence reduces the number of seeds harvested. The number of twists along 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 indices of pod dehiscence. As expected,

the alleles from the cultivated parent decreased PDT. For PDR1W, PDR2W and PDR4W, there were considerable differences in the percentage of shattered pods between the parents (Isemura et al., 2012).

Pod dehiscence (shattering) is essential for the propagation of wild plant species bearing seeds in pods but is a major cause of yield loss in legume and crucifer crops. Although 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 defective, having a premature stop codon. The functional gene, *Pdh1*, was highly expressed in the lignin-rich inner sclerenchyma of pod walls, especially at the stage of initiation in lignin deposition. Comparisons of near-isogenic lines indicated that *Pdh1* promotes pod dehiscence by increasing 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 possibly be useful for crop improvement (Funatsuki et al., 2014).

Common bean (*Phaseolus vulgaris* L.), the most important legume crop, developed a methodological pipeline that comprises a thorough characterization under field conditions, including also the chemical composition and histological analysis of the pod valves. The pipeline was developed based on the assumption that the shattering trait itself can be treated in principle as a “syndrome” (i.e., a set of correlated different traits) at the pod level. Characterized a population of 267

intro-gression lines with objectives: (1) to dissect the shattering trait 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 the chemical composition and/or the histological characteristics of the pod valves; and (3) to test the associations between shattering and other plant traits. Results revealed high shattering levels can be achieved in different modes; shattering resistance is mainly a qualitative trait; and high shattering levels is correlated with high carbon and lignin contents of the pod valves and with specific histological characteristics of the ventral sheath and the inner fibrous layer of the pod wall. Shattering comes with a “cost,” as it is associated with 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 a 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 shattering phenotyping (Murgia et al., 2017).

Loss of pod shattering is one of the most important domestication-related traits in legume crops. The non-shattering phenotypes have been achieved either by disturbed formation of abscission layer between the valves, or by loss of helical tension in sclerenchyma of endocarp, that split open the pods to disperse the seeds. During domestication, azuki bean (*Vigna angularis*) and yard-long bean (*Vigna unguiculata* cv-gr. *Sesquipedalis*) have reduced or lost the sclerenchyma and thus the shattering behavior 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).

Pod dehiscence is a key trait in legumes due to its relevance for seed dispersal and yield losses. In chickpea (*Cicer arietinum* L.), the identification of major and minor genes controlling pod dehiscence is very important when wild genotypes are used to introgress germplasm in cultivated ones. Characterized phenotypically a RIL population from an interspecific cross and used a candidate gene approach to identify orthologous to dehiscence-related genes. The segregation pattern in the RIL population suggests that the trait is under oligogenic control. Through genome mapping and sequencing, developed DNA markers and identified the PDH1 gene as an important regulator of pod shattering in chickpea. Results may help 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).

### **STRENGTHENING OF THE DEHISCENCE ZONE**

A search among field populations of *Lupinus angustifolius* L. and *L. digitalis* Forsk. yielded two morphologically and genetically distinct lines in each species with markedly reduced pod-shattering at maturity. In all four lines, reduced

shattering was found to be due to a single recessive gene, the two genes of each species being non-allelic and probably unlinked. Double homozygotes were obtained, and proved to be fully non-shattering in both species. The anatomical changes resulting in reduced- or non-shattering are of at least two types. In one type of each species there is fusion of the normally divided strips of sclerenchyma in the pod seams, similar to that in the non-shattering Strain 3535A of *L. luteus*. In the others there is a weakening of the sclerified 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).

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 trait lies in the 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 promotes the significant thickening of FCC secondary walls by expression at 15-fold the level of the wild allele, which is attributed to functional disruption of the upstream repressor. Strong artificial selection of *SHAT1-5* has caused a severe selective sweep across ~116 kb on chromosome 16. This locus and regulation mechanism could be applicable to legume crop improvement. It was found 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 through repressor disruption. This regulatory change is correlative with strong artificial selection of *GmSHAT1-5* during soybean domestication with hitchhiking effect on closely linked loci across ~116 kb in chromosome 16 of the soybean genome. This mechanism is distinct from the one underlying grain shattering resistance of domesticated cereals (Dong et al., 2014).

In Common vetch (*Vicia sativa* L.), one of the most important annual forage legumes globally because of its multiple uses and high nutritional content. However, when it matures, the pod dehiscence can cause severe loss of seeds. In this research, used eight shatter-susceptible vetch accessions and 16 shatter-resistant vetch accessions, which were evaluated and selected from 541 accessions, to compare and analyze the influencing factors related to pod dehiscence. Found that the shatter-susceptible vetches all have abscission layers and that the shatter-resistant vetches all lack abscission layers. External valve margin cells, which have not been reported in other plants to date, were located externally to the junction of the fruit valve where the valve margin exists, with the abscission layers below in the ventral suture. It was found that shatter-resistant vetches have significantly thicker external valve margin cell walls and obviously fewer pod wall torsion laps than shatter-susceptible vetches, and there was no significant difference in the pod thickness to width ratio. Confirmed, abscission layers, external valve margin cells, and pod wall torsion laps are the main factors affecting pod dehiscence.

Thus, this research lays the foundation for studying the mechanism of vetch pod dehiscence (Dong et al., 2017).

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 with 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 showed a reduced twisting of the seed pod. The number of twists/cm in the pods was  $0.371 \pm 0.018$  in the *rps1* mutant, which was less than half of the wild type ( $0.866 \pm 0.022$ ). Interestingly, the *isi1*, one of the mutants of seed imbibitions, also exhibited slightly reduced shattering rate ( $73.99 \pm 18.53\%$ ) and number of twists/cm ( $0.579 \pm 0.093$ ). Other mutants were also slightly reduced in number of twists/cm, but their shattering rate was 100%. 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. Thus, 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 improving 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).

### THE ABSENCE OF POD WALL FIBER

In a study conducted to assess the genetic control of the domestication syndrome in common bean (*Phaseolus 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

to be concentrations in three genomic regions with a major effect on the syndrome, 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 gene pool (Koinange et al, 1996).

Hairy vetch, *Vicia villosa* (Roth), is a cover crop that does not exhibit a typical domestication syndrome. Pod dehiscence reduces seed yield and creates weed problems for subsequent crops. Breeding efforts aim to reduce pod dehiscence in hairy vetch. To characterize pod dehiscence in the species, we quantified visual dehiscence and force required to cause dehiscence among 606 genotypes grown among seven environments of the United States. To identify potential secondary selection traits, we correlated pod dehiscence with various morphological pod characteristics and field measurements. Genotypes of hairy vetch exhibited wide variation in pod dehiscence, from completely indehiscent to completely

dehiscent ratings. Mean force to dehiscence also varied widely, from 0.279 to 8.97 N among genotypes. No morphological traits were consistently correlated with pod dehiscence among environments where plants were grown. Results indicated that visual ratings of dehiscence would efficiently screen against genotypes with high pod dehiscence early in the breeding process. Force to dehiscence may be necessary to identify the indehiscent genotypes during advanced stages of selection (Kissing Kucek et al., 2020).

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

In other members of the Fabaceae tribe, domestication has successfully eliminated PD (e.g. *Pisum* sp.) or reduced 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.), the transcription factor *SHAT1-5* and the gene *Pod dehiscence 1 (Pdh1)* mediate and control PD. In common bean (*Phaseolus vulgaris* L.), various QTL have been identified among bean races, the most documented being the *Stringless (St)* gene in snap beans (Petr Smýkal et al., 2015).

PD is influenced by environmental conditions, length of 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 pods to standardize moisture and/or applying force to a pod to induce dehiscence were more associated with genetic effects than measuring PD under field conditions. Such methods, particularly measuring the force needed to induce dehiscence, demand substantial phenotyping time and specialty equipment. Identification of traits that are easier to measure and highly correlated with PD could improve breeding efficiency. Such secondary selection traits could accelerate improvement of hairy vetch. Wide variation in visual and force to dehiscence existed among diverse genotypes. More importantly for selection, multiple lines exhibited indehiscence or very low levels of dehiscence.

This dataset also demonstrated environmental influence on PD, which is well documented in other species. Growing environment contributed substantial amounts of variance for visual dehiscence and force to dehiscence. Moreover, correlations between metrics of PD, pod morphology, and flowering maturity significantly differed among environments. To separate genetic effects from environmental influences and interactions, PD studies should utilize multiple environments. Secondary selection traits to speed phenotyping



would need to consistently correlate among diverse environments within a breeding program region of interest.

Spiraling was highly correlated with PD and was a high-throughput measurement, requiring only 15 seconds per sample. Visual dehiscence provided higher resolution in PD than spiraling and was moderately time intensive, requiring 5 min to rate per line, at 50 pods evaluated per line. Force to dehiscence was the most involved measurement, requiring 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 strongly dehiscent lines, force to dehiscence may be useful for identifying extreme lines most resistant to dehiscence. For initial screenings of dehiscence, spiraling could identify the genotypes most susceptible to PD at low cost. Visual dehiscence would be 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).

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

and pith tissue would not be useful PD secondary selection traits for breeding programs including cold temperate climates.

Further study is needed to understand the physiology of pith tissue in hairy vetch pods. The pith tissue created a foam-like structure that seemed to inhibit compression force from breaking a pod, hence the trait's contribution to force to dehiscence. However, the pith tissue may not be 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, the trait of pith tissue could serve as a covariate when analyzing force to dehiscence.

The fracture structure of the 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 pod wall fibers at an angle to pod sutures, which can cause spiraling of the carpel. As the evaluation of 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 traits showed inconsistent correlation with PD metrics among environments, such as pod flexibility. Such traits would not be reliable secondary selection traits for PD. Pod moisture was not correlated with visual dehiscence or force to dehiscence. Consequently, pods in our study had likely reached the critical pod moisture required for PD. The weak correlation between spiraling and pod moisture could indicate that some samples were above the critical pod moisture threshold for PD. Moisture

contents in our evaluation (6.7% to 9.3%) were below the critical pod moisture (10.1% to 10.4%) associated with PD in soybean. However, these moisture contents were above the stable moisture found in common vetch (5%). Results of PD after various pod drying times, heat conditions, and pod moistures to identify critical pod moisture in hairy vetch.

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

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

#### MAJOR REDUCTION IN SUTURE FIBER

Snap bean (*Phaseolus vulgaris* L.) breeding programs are tasked with developing cultivars that meet the standards of the vegetable processing industry and ultimately that of the consumer, all the 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 dry bean, snap bean immature pods and seeds are consumed as a vegetable. Several pod traits are 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 round pod cross section, and pods tend to be longer with cylindrical seed shape. Seed color is an important trait in snap bean, especially those used for processing, as processors prefer white-seeded cultivars. RR6950, a small seeded brown indeterminate type IIIA dry bean accession, was crossed to the Oregon State University (OSU) breeding line OSU5446, a type I Blue Lake four-sieve breeding line to produce the RR138 F<sub>4:6</sub> recombinant inbred (RI) mapping population. The RR138 population was genotyped with the BARCBean6K\_3 Beadchip, and single nucleotide polymorphisms (SNPs) were used to assemble a linkage map, and identify QTL for pod traits. The map was populated with an average of one SNP per 1.4 cM, spanning 11 linkage groups. Seed and flower color 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 total genetic variation. QTL for a suite of important processing traits including 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).

In snap bean, pod wall fiber and pod suture fiber are separate traits, and there has been some question as to

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

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

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