

Brassica shatter-resistance research update

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ABSTRACT

An overview of research into shattering and shatter resistance is presented in the context of recent advances in molecular genetics of the trait in *Arabidopsis*. Several methods of testing for shatter resistance have been devised, but procedures that directly measure intrinsic pod strength of the variety, such as the pendulum test appear to be more satisfactory. Previous observations on the anatomical basis of shattering have been further confirmed and there is evidence for enzymatic degradation of the abscission layer in susceptible varieties. Similar to the previous research in *B. rapa*, recent molecular genetic research in *Arabidopsis* has shown involvement of 3 loci in shattering and epistatic relationships between them. Several Australian canola varieties possess substantial tolerance to field shattering but further improvement is needed to avoid the need for windrowing and to allow direct heading. The genes discovered in *Arabidopsis* provide transgenic approaches to this task. Work is in progress to transfer shatter resistance genes from *B. juncea* and *B. rapa* to *B. napus* by conventional breeding methods.

Key words: shatter resistance - anatomical basis of shatter resistance - pendulum test – sarson - interspecific hybridisation.

INTRODUCTION

Resistance to shatter is an important trait for canola improvement in Australia because the crop ripens and is harvested under hot and often windy summer conditions. Shattering (dehiscence) involves detachment of the pod valves (Figure 1), which enclose the seed, from the replum. It could occur in ripe standing crops under windy conditions due to impact from other plants and in windrows from the impact of harvest machinery. Shatter-resistant canola varieties could be direct headed avoiding the cost and inconvenience of windrowing the crop.

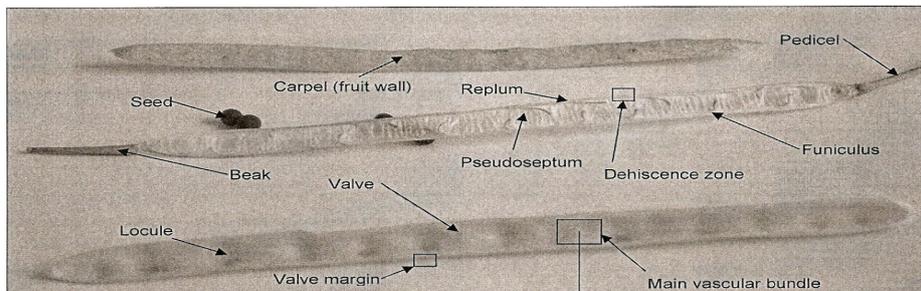


Fig. 1. Structural features of a *B. napus* pod (Agius et al. unpublished).

ANATOMICAL AND PHYSIOLOGICAL BASIS OF SHATTERING

Kadkol et al. (1986a) showed the occurrence of an abscission layer, consisting thin walled and non-lignified cells, in the sutures of siliquae of shatter-susceptible *Brassica* and the absence of the abscission layer in the shatter-resistant *Brassica rapa* types, *yellow sarson* and *brown sarson* (Figure 2). These abscission layers predispose pods to shattering.

Research in *Arabidopsis* has shown that development of the abscission layer is brought about by reduced auxin levels in the dehiscence zone by a gene known as IND. Mutant *ind* genotype prevents differentiation of tissue in the dehiscence zone into an abscission layer resulting in indehiscent fruits (Sorefan et al., 2009) much like the *sarsons*.

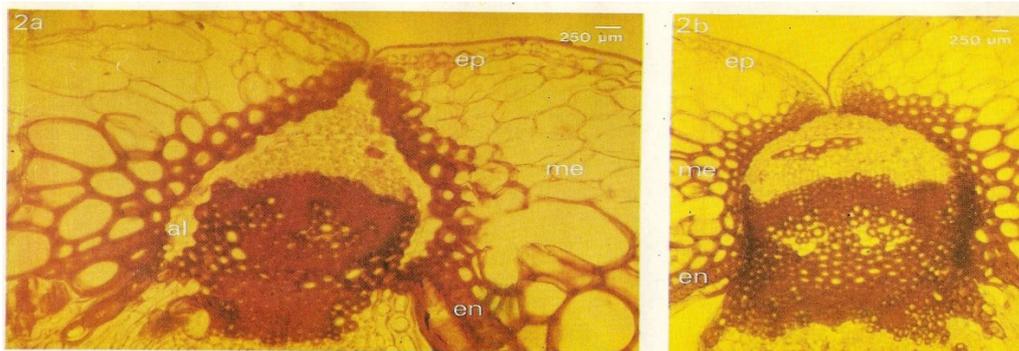


Fig. 2. Transverse sections (x120) through dehiscence zones of fresh pods of shatter-susceptible *B. napus* cv. Midas (2a) and shatter-resistant *B. rapa* var. *brown sarson* cv. DS-17-D (2b) stained with phloroglucine (al = abscission layer, en = endocarp, me = mesocarp, ep = epicarp) (from Kadkol et al. 1986a).

There is strong evidence in *B. napus* for enzyme action (beta1,4 glucanase) in the abscission layer resulting in degradation of the middle lamella (Meakin and Roberts, 1990a and b)). It is likely that the types with intermediate levels of shatter resistance might be associated with low levels of enzyme activity in the abscission layer such that, although non-lignified, the strength of the intact tissue is adequate to prevent significant levels of shattering and seed loss.

ACTIVE OR PASSIVE DEHISCENCE?

Active dehiscence involves development of stresses in the drying pods due to an in-built mechanism ultimately leading to dehiscence or shatter without the need for any external disturbance. Pods of even the most shatter-susceptible canola varieties, despite the weakening of the abscission layer still need some external mechanical disturbance to produce shattering (Kadkol et al. 1986a, Meakin and Roberts, 1990a). It is possible to store intact pods from such varieties in vials for long periods. Also, in contrast to pods of actively dehiscing plants, it was not possible to induce dehiscence in canola pods by manipulating humidity (Kadkol, unpublished data). These observations are consistent with a passive dehiscence mechanism with no significant forces arising within the pod valves as the pods ripen and desiccate to overcome the tissue in the separation layer (Kadkol et al., 1986a). There is some evidence for differences in the pattern of dehydration of pods between shatter-susceptible *B. napus* pods and resistant *B. rapa* (Squires et al., 2003) but this is not likely to be adequate to produce active dehiscence similar to that in the pods of Fabaceae.

METHODS FOR EVALUATION OF SHATTER RESISTANCE

Field evaluation for shatter resistance is inaccurate due to varying weather conditions during harvest from one season to the next. Notes on shattering in breeding programs tend to be opportunistic. For this reason laboratory testing for shatter resistance is required. A basic requirement for any laboratory test is that it simulates the process as it occurs under natural conditions. Most external forces acting on the pods leading to dehiscence would be bending forces acting at the base of the pods resulting in the valves separating from the replum along the dehiscence zone. Because of this the strength of the tissue in the dehiscence zone is logically the key trait that determines the level of shattering.

The cantilever test (Kadkol et al., 1984) which was subsequently developed into a simpler and inexpensive pendulum test (Liu et al., 1994) took the natural process of dehiscence into consideration by testing the pod as a cantilever. The pendulum method (Figure 3) provided a further improvement in simulation of the natural process of shattering as it is a dynamic test that achieves rates of loading comparable to those in the natural dehiscence process in the field. Pod strength assessed using cantilever tests was significantly correlated ($r = 0.59$) with shattering in the field assessed as percentage of shattered pods on the main stem and it was

the only trait showing substantial correlation with percentage shattering (Kadkol et al., 1984). The pendulum test (Figure 3) provided results that were correlated strongly with those from cantilever test (Liu et al., 1994) and also with estimates of shattering in the field ($r = 0.86$, Wang et al., 2007).

Many of the laboratory tests published to date appear to ignore the above principle and thus provide a general test of pod strength. The random impact test (RIT) is an example and this involved shaking pods together with ball bearings in a container using a mechanical shaker (Bruce et al., 2002). The number of pods that remained intact was taken as a measure of shatter resistance. The authors did not discuss the correlation between results of these tests with field shatter but Wang et al. (2007) compared the degree of correlation between field data and results from pendulum test and RIT. RIT showed a lower level of association with field shatter ($r=0.59$) than the pendulum test.

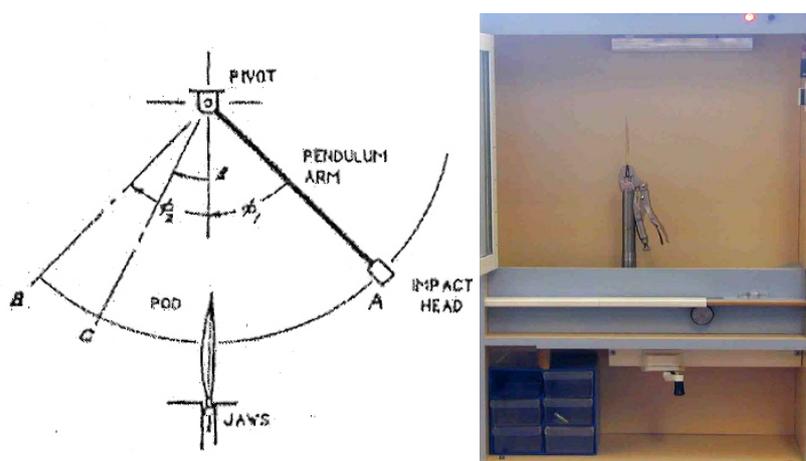


Fig.3. Arrangement and analysis of pendulum (from Liu et al. 1994) and the new pendulum machine for testing canola pods.

IMPROVEMENTS TO THE PENDULUM MACHINE

The original pendulum testing machine designed by Liu et al. (1994) has been improved (Figure 3) to include protection from air currents and capture of seed from the tested pods. Measurement of pod length is carried out electronically and the point of impact on the pod in relation to its length is standardised. Electronics of the machine have been improved to simplify data logging. Software enhancement has provided compatibility with the modern 32-bit computers and a direct interface through Excel.

GENETICS OF SHATTER RESISTANCE

Due to lack of genetic variation for shatter resistance in *B. napus* Mendelian genetic studies of shatter resistance have been restricted to *B. rapa*. Shatter resistance in *B. rapa* var. Brown Sarson and var. Yellow Sarson is determined by 2-3 genes in crosses with shatter-susceptible cv. Torch (Kadkol et al. 1986b). The F_2 segregation pattern showed a dominant epistatic interaction. This was consistent with results of a quantitative genetic analysis of one of the crosses, Torch X DS-17-D, which showed significant non-additive and additive genetic variance and a high broad sense heritability (Kadkol et al. 1986c). These results were further confirmed by Mongkolporn et al. (2003) using the pendulum machine for shatter resistance assessments in a subsequent study.

Molecular genetic research in *Arabidopsis* has resulted in discovery of mutants that show altered pod anatomy in the dehiscence zone. Two closely related MADS-box genes, SHATTERPROOF1 (SHP1) and SHATTERPROOF2 (SHP2) produce a dehiscent phenotype. The double recessive *shp1shp2* produces indehiscent fruits that do not possess an abscission

layer in the dehiscence zone and also show reduced lignification of the valve margin cells (Liljegren et al., 2000). Rajani and Sundaresan (2001) discovered the ALCATRAZ (ALC) gene which is involved in the development of the abscission layer in the dehiscence zone. The mutant *alc* phenotype does not dehisce and does not possess an abscission layer. This gene is expressed at the valve margin and it appears to act independently from the SHP and FUL genes. The INDEHISCENT (IND) gene appears to interact with SHP, ALC and FRUITFULL (FUL) genes to determine tissue differentiation and fruit patterning. Also, the FUL gene is required to restrict the expression of IND to valve margins and does not have direct role in shattering (Liljegren et al. 2004). Further research has shown that IND influences lignification of the valve margins and differentiation of the abscission layer by regulating auxin levels (Sorefan et al., 2009).

The direct role of SHP, ALC and IND genes in determining the shatter phenotype of *Arabidopsis* plants corresponds well with the previous reports of 2-3 genes involved in determining shatter resistance in *B. rapa* and interacting epistatically by Kadkol et al. (1986b) and Mongkolporn et al. (2003). The anatomical effects of *alc* and *ind* mutants are similar to the anatomical features of the naturally occurring shatter-resistant *B. rapa* var. *sarson* types described above and used in genetic studies by Kadkol et al. (1986b and c). It is likely that this similarity could extend to the biological processes and genes controlling shattering in *B. napus*.

MOLECULAR MARKERS FOR SHATTER RESISTANCE

Development of molecular markers for shatter resistance in *B. napus* is restricted due to lack of variation for this trait in the species. However, markers could be developed in related species such as *B. rapa* and applied to breeding populations developed from interspecific crossing for transferring the trait to *B. napus*. Mongkolporn et al. (2003) identified three RAPD markers in *B. rapa* using Torch X DS-17-D crosses previously studied by Kadkol et al. (1986b). Two of the markers cosegregated with recessive alleles, *sh1* and *sh2* and the third marker cosegregated with the dominant alleles at both the loci. Further work is needed to refine these into robust markers.

BREEDING CANOLA FOR SHATTER RESISTANCE

As an indirect selection method for tolerance to field shattering some Australian breeding programs (e.g. Canola Alliance and Nuseed) have simply avoided windrowing and have practised direct heading of breeding trials and plots. Whilst the varieties from these programs have not been properly characterised for shatter resistance, there appears to be substantial improvement in field shatter tolerance in them relative to older varieties. However, further improvement is required to avoid the need to windrow. The traditional approach to breed canola for such higher levels of shatter resistance is based on interspecific hybridisation or resynthesis of *B. napus* using shatter-resistant species in U's triangle.

Prakash and Chopra (1990) carried out interspecific hybridisation between *Brassica juncea* and *B. napus* and were able to isolate a reconstituted *B. napus* plant with complete non-dehiscence of its fruits. This plant had normal meiosis and formed 19 bivalents. Its seed fertility improved to 84% from the original low (23%) levels. Agnihotri et al. (1990) attempted to transfer shatter resistance from *Raphanus* into *B. napus* using *Raphanobrassica* as the bridging material. This resulted in material with variable fertility. Interspecific hybridisation with *B. rapa* var. *Brown Sarson* and var. *Yellow Sarson* (Kadkol et al. 1991) has been promising in initial results but further work is required to fully characterise and assess the shatter-resistant selections for meiotic stability and agronomic traits. In a Canadian study, lines derived from complex crosses made for development of yellow seeded canola showed better shatter resistance than standard Canadian *B. napus* varieties (Wang et al. 2007). However, an Australian variety, cv. Range matched the best shatter-resistant lines in this study. This could be explained on the basis that cv. Range was selected indirectly for shatter resistance by direct heading of the breeding plots at all stages of selection as described above. Also, many Canadian varieties are quite susceptible to shatter (at least under Australian conditions).

An example of resynthesis of *B. napus* to create variation for shatter resistance is provided by Summers et al. (2003) who studied a line, DK142, derived from resynthesizing *B. napus* using *B. oleracea alboglabra* and *B. rapa chinensis*. DK142 showed superior shatter

resistance assessed using the RIT method at all locations but appeared to have significantly lower levels of seed set relative to the commercial variety, Apex.

Several transgenic approaches for producing shatter-resistant *B. napus* using the *Arabidopsis* genes described above (e.g. Vancanneyt et al, 2003) and genes involved in enzymatic degradation of the separation layer are available (e.g. Roberts et al. 2000, Ogawa et al., 2009) for transformation. Chandler et al. (2005) transformed winter *B. napus* cv. Erox and spring type cv. Drakkar using *S. alba* MADSB gene to produce shatter-resistant plants. The transformed plants possessed altered dehiscence zone anatomy wherein the valve margin cells were not lignified. In these studies the transformed plants were not assessed for agronomic traits.

CONCLUSIONS

There has been rapid growth in molecular genetic research shatter resistance in *Brassicas* in the last decade. The biological process and the genes controlling it are better understood and we have good methods to screen breeding material for shatter resistance giving us the necessary tools for transferring the trait to *B. napus*. Interspecific crosses to date have been associated with reduced fertility possibly due to meiotic instability. Further work is required to overcome these problems. Transgenic approach is promising but the genes are all patented and the comparative agronomic data from the transformed napus plants has not been published.

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