PROJECT REPORT No. 323

POD SHATTER RESISTANCE IN OILSEED RAPE: GENETIC CONTROL; BREEDING TO DEVELOP RESISTANT VARIETIES

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POD SHATTER RESISTANCE IN OILSEED RAPE: GENETIC CONTROL; BREEDING TO DEVELOP RESISTANT VARIETIES

by

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CONTENTS

Abstract

Fully mature pods of oilseed rape (*Brassica napus* L.) are extremely sensitive to opening resulting in seed loss (pod shatter). This can take place prior to harvest owing to disturbance of the canopy by wind or during harvesting as the combine machinery moves through the crop. Typical losses vary between 8-12% of the potential yield but reductions of up to 50% have been estimated in seasons when weather conditions were poor prior to and during harvest. Reduction in the sensitivity of pods to opening would increase the proportion of the yield recovered by the combine and thereby improve production efficiency.

Little variation in shatter susceptibility is thought to be present in current commercial breeding lines but sources of resistance to pod shatter have been sought amongst related species. More resistance is apparently present amongst some lines of *B. juncea, B. carinata* and *B. nigra* as well as amongst the putative parents of *B. napus* (*B. oleracea* and *B. rapa*). Attempts to introgress shatter resistance traits from related species into *B. napus* can be complicated by linkage with unwanted characters. However, synthesis of new *B. napus* lines derived from interspecific crosses between *B. oleracea alboglabra* and *B. rapa chinensis* has provided a range of useful variation amongst which increased shatter resistance has been identified. One of these high shatter resistant lines, DK142, developed at the JIC, had been shown through prelimiary studies to be substantially more resistant to shattering than conventional material.

The current work established that shatter resistance in DK142 was environmentally stable at locations in W & E England and Belgium. There were a wider range of plant-to-plant shatter values in DK142 than in Apex but the average resistance was three times greater in the synthetic line than in the commercial line.

Resistance correlated with parameters of mature pod size, but the characters were defined too late to use them as accurate predictors of high shatter resistance in individual plants. Resistance is heritable and was recoverable in F2 lines together with the dehiscence zone (DZ) characters that we determined underpin resistance in DK142. The main feature responsible for increased pod shatter resistance was shown to be an increase in the size and energy required to break the main vascular bundle entering the pod valve from the pedicel. Although some increased shatter resistance in the parental material can be attributed to a higher area of DZ it has also been shown that reduced cell separation is not a mechanism leading to increased shatter resistance in this material.

However, these traits which are visible under the microscope, can only be measured in fully developed pods. This precludes their direct use as a predictor trait early in plant development. Thus, the need for a genetic marker is of paramount importance for the identification in breeding programmes of individuals with high shatter resistance values. Microsatellite, RFLP and AFLP

markers were successfully associated through QTL and association mapping with several factors contributing to increased pod shatter resistance.

Breeding towards the integration of pod shatter resistance into competitive varieties of oilseed rape was shown to be possible, although the existence of several deleterious traits in the parental material indicates that considerable breeding effort will be required in order to restore an acceptable genetic background together with the factors conferring pod shatter resistance; the results of this programme will greatly facilitate this process.

Summary

The foundation for this work was laid in the MAFF-funded project NFO306 'Fracture analysis of oilseed rape pods' in which a procedure (Random Impact test or RIT) was devised in order to identify the most resistant lines amongst resynthesised *B.napus* lines produced at the John Innes Centre. The RIT procedure was designed to mimic the random impacts received by pods during harvesting and assess shatter susceptibility in standard conditions. This identified several resynthesised lines which were significantly more resistant to shattering than current commercial cultivars. One of these, DK142, is derived from a selfed, doubled haploid, microspore-cultured plant that was produced from a cross between a doubled haploid winter breeding line and a synthetic interspecific hybrid of wild *B. oleracea alboglabra* and *B. rapa chinensis.* Anatomical analysis suggested that changes in the structure of the pod dehiscence zones (DZs) might be responsible for the increased resistance to valve separation that in susceptible lines results in the release of the seeds (shatter).

One of the most important conclusions drawn from this MAFF-funded project was to establish the main features of the genetic basis of the psr trait. Results indicated that resistance to shattering in oilseed rape is clearly a complex, quantitative trait, most likely under the control of more than a few genes, acting together to produce the level of shattering observed on mature plants. A further level of complexity was also evident in that, as well as being complex in the genetic sense, the psr trait is also complex in the sense that it is contributed to by a number of factors concerning pod structure and anatomy, as revealed by the work at LARS and SRI. It was concluded that genetic studies on these component traits, and possibly their genetic control and subsequent manipulation in breeding programmes or marker-assisted breeding, is likely to be somewhat easier and more effectively directed than dealing with the psr trait per se. **These findings from this previous work formed the focus for studies in the SAPPIO Link project reported here, and helped to shape the format and work programmes.**

The first objectives of the anatomical and mechanical work in the current project were to confirm the shatter resistance of DK142 by comparison with the commercial cultivar Apex and to determine its environmental stability at sites in the UK (IACR-Long Ashton, Bristol and JIC, Norwich) and Belgium (Bayer Crop Science, Gent).

Environmental stability of shatter resistance in parent lines

Seed was collected in 2000 from self-pollinated, second-generation DK142 plants grown at Long Ashton which were derived from the original microspore plant. This was distributed together with seed of the commercial cultivar Apex, for sowing by each of the project partners. Sowing times and subsequent growing-on conditions were varied from site to site during November and December 2000 in order to bring plants to the four-leaf stage during January ready for planting 0.5m apart in double rows outside, prior to stem extension at all three sites during March. As a result of this 'early' sowing all pods were fully ripe by early August but weather conditions delayed collection of pods for analysis until September. In all environments at least 100 plants of DK142 and 50 of Apex were collected for RIT analysis.

In Gent and Long Ashton, a second 'late' sowing was made in February 2001. Seedlings were artificially vernalized and transplanted in the field at Gent in June. Fully mature pods were collected in October. At Long Ashton, plants transferred to pots in May were grown in a glasshouse and were also fully ripe in October.

The Random Impact Test (RIT) was adapted for use in this project as follows. The moisture content of fully mature pods collected from individual plants was brought to a uniform and constant level by placing them for at least 3 days at 25°C and 50% RH in a controlled environment cabinet. Twenty undamaged pods per plant were then placed together with six steel balls of 12.5-mm-diameter in a 20-cm-diameter cylindrical container. The container was mechanically shaken at a frequency of 4.98 Hz and a stroke length of 51 mm for two 10 s periods, followed where required, by one period each of 20, 40 and 80 s. The times were chosen to give equal intervals after log transformation. At the end of each period, pods were examined and classed as shattered if at least one of the valves had detached. A standard statistical procedure using the 'GenStat' package (Lawes Agricultural Trust, IACR-Rothamsted) enabled the calculation of the time (s) taken for 50% of the pods to shatter (RIT_{50}).

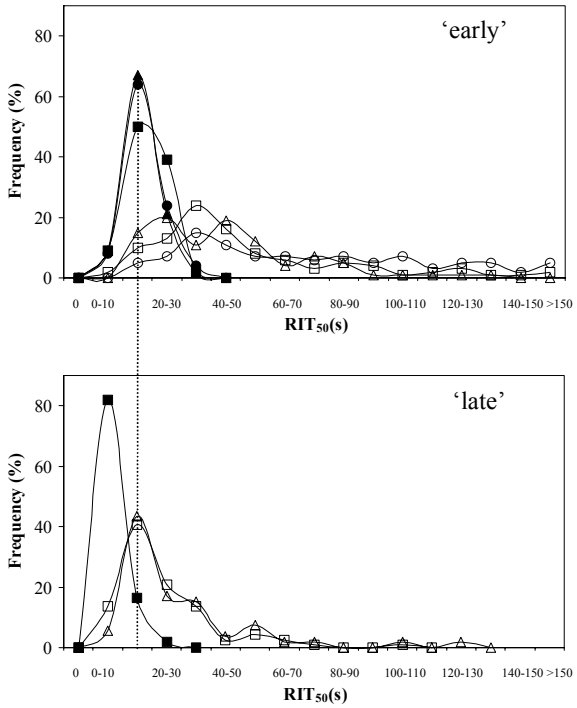

Fig. 1. Distribution of shatter resistance (RIT_{50}) of Apex (closed symbols) and DK142 (open symbols) populations grown during 2001 from 'early'- and 'late'-sowings at Bristol (squares), Norwich (circles) and Gent (triangles). Note the increased susceptibility of 'late'-sown populations.

A range of shatter susceptibility values were recorded within populations of Apex and DK142 (Fig. 1). In populations of Apex, the range of resistance was narrow and distributed around a mean of 18 s. In contrast, the resistance of DK142 was on average three times greater than Apex and the range of shatter values much wider. **Significantly higher RIT_{50} values were therefore confirmed in DK142 compared with Apex and RIT_{50} distribution patterns within each line were similar at all sites. This clearly demonstrated the absence of environmental influence on shatter resistance in both the commercial and the synthetic line**.

By contrast, the time of sowing had a significant effect on shatter susceptibility in populations of both lines. In both 'early-' and 'late-sown' populations there was little site-to-site variation in mean values for DK142 which were consistently three times greater than for Apex. However, average shatter resistance of all 'late-sown' populations for both lines was approximately half that of 'early-sown' populations. It seems likely that the shorter growth period for plants in the 'late' sowings resulted in the accumulation of lower assimilate levels and smaller plant biomass than in the 'early' sowings. This is clearly in accord with established agronomic knowledge of the effects of sowing time on yield levels and the levels of shatter susceptibility in 'winter-sown' as opposed to 'spring-sown' oilseed rape. However, it is important to remember that relative shatter values between the shatter susceptible commercial cultivar and the more resistant synthetic line remain the same indicating that underlying differences in the mechanism of resistance also remain the same.

Morphological differences between Apex and DK142

Flower abnormalities in DK142 included delayed and reduced pollen production. Seed numbers and weight per pod were less than 50% compared with Apex (11 seeds weighing 52 mg in DK142 compared with 23 weighing 116 mg in Apex). These differences did not appear to originate from differences in ovule numbers, which were similar in the flowers of both lines. We also noted that line DK142 had a tendency to set fewer pods. Investigations into the relationship between pod numbers and shatter resistance showed that mature pods from a sub-set of plants that had been pruned 5 weeks after anthesis, were approximately 50% more resistant than on unpruned plants of both DK142 and Apex. Pods from pruned plants weighed more than pods from unpruned plants and in both lines pruning increased the weight of the pod receptacle more than the weight of the seed. The extra pod receptacle weight was mainly a result of the septum and valves being heavier per unit length. Thus, the reduced seed and/or pod set in this line may be responsible for increased partitioning to the receptacle and may be a causal factor helping to determine increases in shatter resistance. However, as pods from pruned DK142 plants were still more resistant than those from Apex with a similar number of pods, this supports the likelihood that increased shatter resistance in DK142 pods is also genetically determined.

The next priority was to determine the descriptive statistics of DK142 and Apex pods in order to identify, if possible, associated or linked characters that might enable the early recognition of shatter resistant plants in breeding populations. The very large difference in shatter resistance values of Apex and DK142 strongly indicated a genetic component and the most marked difference between pods from the two parents was the weight and size of the receptacle. DK142 pods had thicker pedicels, pod walls, septa and beaks and despite DK142 pods being 15% shorter than Apex, the valves were more than 25% heavier and the beak plus septum more than 50% heavier. However, total pod weight of DK142 was 23% lighter, because its seed weight was 55% less due to 50% fewer seeds. Indeed, the partitioning ratio of seed to receptacle in DK142 (0.6:1) contrasted strongly with Apex (1.7:1) where the weight distribution was clearly partitioned towards the seed. **All pod parameters correlated significantly with shatter values (RIT_{50}) and some gave a greater than 50% chance of accurately predicting shatter resistant pods. However,**

these characteristics could be determined accurately only at maturity. This precludes their use as a predictive morphological marker for the early identification of shatter resistant plants in a breeding programme.

Heritability of resistance in plants derived from crosses between DK142 and Apex.

The distribution pattern of shatter value was similar in the F1 and Apex populations whereas that of the F2 population closely resembled DK142. This suggested the recessive nature of the shatter resistance trait and clearly confirmed its heritability, indicating that the genes and characters associated with increased shatter resistance are present in the populations of derived F2 plants. Within line variation of pod characters associated with resistance in the parent lines was monitored in the F1 and F2 populations to establish which particular parameters determined shatter resistance. In all populations, including those with all or the majority of plants falling within a small range of resistance, dimensions of the pod receptacle correlated positively with shatter. Variation in valve and beak plus septum weight produced the most significant correlations, closely followed by valve length and thus septum length. Both parents appear to have contributed positive characters i.e., long pods from Apex and a high weight/unit length from DK142. Thus, the range of resistance in the F2 population was expected to exceed that found in DK142 and this was indeed the case.

Shatter resistance in the F2 population was more than double that of Apex, and seed numbers and weight per pod were also improved compared with DK142. Indeed, seed weight per pod was only ~10% less than for Apex. This is agronomically encouraging as the evidence so far suggests that restoring normal fertility should be compatible with retaining the enhanced shatter resistance.

Anatomical analysis

In all populations, the weight of the septum and of the valves each correlated strongly with resistance. These two components of the pod are joined by a dehiscence zone (DZ) which is located in sutures that extend on each side and along the length of the two valves. The DZ contains two or three rows of simple, thin-walled cells that separate the heavily-thickened valve edge cells from the vascularized replum of the septum. During the later stages of pod senescence, approximately 7 weeks after anthesis, the thin-walled cells separate. We believe the weight of the valves and the septum can affect resistance because they determine the width and properties of the DZ.

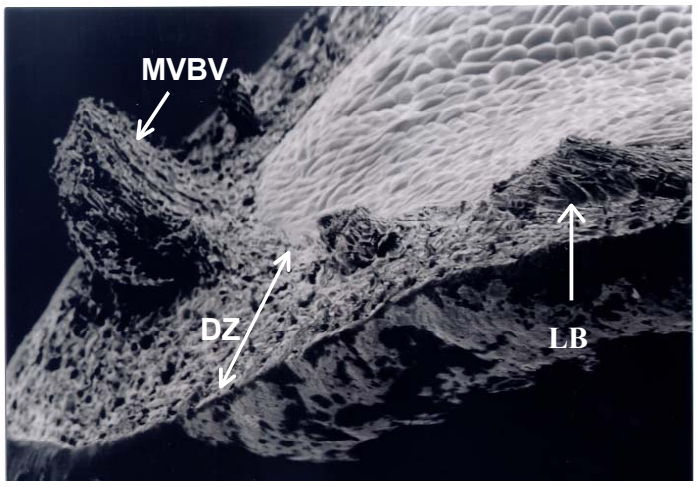

Fig. 2. Section of fully mature pod from the pedicel end of the valve viewed under the SEM.

The structure of the DZ and in particular the architecture and spatial distribution of the vascular tissue at the valve edge were determined in fully developed, green pods embedded and examined under a high-power microscope. From this examination the orientation and size of the main vascular bundle of each valve (MVBV) appeared different in the two parental lines, with the MVBV larger and angled more longitudinally in DK142. This tissue passes from the pod stalk (pedicel) through the thin-walled cells of the DZ and into the valves, forming connecting 'bridges' of vascular tissue. In addition, vascular bundles close to the MVBV could be seen passing between the replum of the septum and the valve and the number and angle of these bundles also differed between Apex and DK142. At full pod maturity the valves remain attached to the replum by this vascular tissue that passes from the pod wall through the dissociated cells. Separation can only take place, releasing the seeds, when these connections have been broken as a result of impact with other pods or with the combine during harvesting.

A scanning electron microscope (SEM) was used to examine the separated surface of the valve edge of mature pods. Dimensions of the vascular tissue and the DZ were measured at various positions. The width and breadth of the fractured surface of the main vascular bundle of the valve (MVBV) was also measured and the amount of vascular tissue additional to the MVBV was estimated.

For all DZ characters, the range of values found in Apex was narrower and the mean values lower than those in DK142. The variation of many of the DZ features correlated significantly with the variation in shatter scores, in particular, the size of the MVBV. The highly significant correlations of this tissue with shatter values in both parent lines and subsequently in F2 and double haploid populations indicated and then established that the size of this tissue plays a key role in shatter resistance. Moreover, in F2 populations where a more detailed analysis of the vascular tissue was carried out, correlations indicated that longitudinal bundles (LS) were important in determining resistance. **If longitudinal bundles are stronger because of their direction, it is likely that the more longitudinally orientated MVBV of DK142 is stronger per unit area than that of Apex.**

Mechanical analysis using a novel micro-fracture test (MFT)

An analysis to establish the amount of energy needed to separate the valve from the replum was carried out using the MFT procedure developed at Silsoe Research Institute. Pod wall tissue was excised with a small, circular saw to isolate areas consisting of the septum and valve, between which the DZ remained intact. These were prepared from the pedicel end of the valve and included the MVBV. After completion of the MFT, the fracture surface of the piece of valve removed during testing was examined under the SEM to establish the area of the DZ and that of the MVBV. This test confirmed the important relationship between MVBV size and shatter resistance, as the force and energy required to break open small sections that were no more than 2% of the entire pod correlated with high significance with shatter resistance of whole pods. Tests on sections further down the pod, where there was no vascular intrusion, confirmed the relationship between DZ width and shatter resistance. For resistant pods, the size of the large MVBV determines the upper limit to pod strength. Weak pods, not only of Apex but including weak pods of DK142 and F2, have a small MVBV and because of this, the width of the DZ contributes to pod strength.

Views of the separated valve surface under the SEM suggested that more tearing at the inner edge of DZ might have taken place in DK142 than in Apex and the microfracture test confirmed that side sections of DK142 required 50% more force per unit area to separate them. This was because the DZ of DK142 was 40-50% wider. However, a similar amount of energy per unit area was required to separate the undifferentiated, simple cells in both lines as the extra number of torn rows of cells in DK142 was in proportion to its wider DZ compared with Apex. Thus, although the wider DZ of DK142 gives additional resistance, between line differences in cell separations are not important. **It seems clear therefore, that the increase in shatter resistance in DK142 is due to the size of its DZ and DZ components particularly the size of its vascular tissue.**

F2 segregation

Segregation data observed from the F2 generation of crosses between DK142 x Apex showed that the pod shatter trait was recoverable. A number of deleterious traits were identified; low seed set owing to partial sterility and aberrant reduced width petals were the more obvious in this generation but F2 progenies with normal flowers and pollen were recovered and some of these did produce mature pods showing normal fertility and also pod shatter resistance. This demonstrated that the pod shatter trait was recoverable in segregating material and further that the most serious defect in the synthetic parental material (poor fertility) are not pleiotropically associated. It may be concluded that further backcrossing of this material to Apex will make progress towards improving the agronomic performance whilst retaining shatter resistance.

Identification and analysis of undesirable traits

The observation of deleterious traits segregating in this material was not unexpected. It was certainly unfortunate that susceptibility to stem canker was segregating but the results suggest that this is under the control of a single major gene and that it is not linked to the target pod shatter resistance. If this is correct then it will prove possible to remove the susceptibility during the introgression process using a combination of selection and further backcrossing. The problem of reduced fertility may indicate an underlying rearrangement within the genome which could be more difficult to deal with. However, there were a good number of lines segregating that have normal fertility and there is no correlation between the fertility and the pod shatter resistance, this indicates that it is unlikely that the gene(s) controlling the pod shatter resistance are involved in any rearrangements. These phenotypic observations leading to a suspicion of the presence of rearrangements may be reviewed when genetic mapping information becomes available.

Progress towards varieties

Progress towards introgression of this trait into commercial breeding material has been slower than hoped owing to the biology of the character. It has not proved possible to identify any associated phenotypes that can be assessed at flowering time or earlier to act that correlate with PSR. It has not, therefore, been possible to avoid treating PSR as a maturity trait and delaying selection until harvest time. The added complication that some degree of segregation for PSR can occur even from a selected individual means that it is necessary

to select and backcross with several individuals of the next generation. Each cycle of breeding requires cross, selfing, segregation and selection under field conditions. This results in a minimum breeding cycle time of 2 years per backcross. Nevertheless from the first cycle of breeding alone it has been possible using selection to fix enhanced pod shatter resistance by F4 with different degrees (up to 5-fold) over the control lines. In this selection scheme we have also been able to reduce, if not eliminate, most of the undesired agronomic penalties originally observed in the F2 population. The value of genetic markers is particularly high for traits of this type since well-defined markers that are co-dominant could in oilseed rape lead to a backcross cycle time of 6 months. Such markers have now been developed and will be available to assist the development of this material into finished varieties.

Genetics and breeding

Two doubled haploid lines of synthetic *Brassica napus*, DK142 and DK129, both produced at the JIC and identified as having significantly higher levels of resistance to pod shatter than average, were crossed to cv Apex to provide two F1 families - POSH1 (from DK142 x Apex) with six individuals and POSH2 (from DK129 x Apex) with four individuals. **These ten F1 individuals formed the basis of the work of the project in two ways:**

1) the ten F1 individuals were selfed to produce a segregating F2 population from amongst which individuals combining high psr with good agronomic characteristics could be selected for breeding work and

2) microspore-derived doubled haploid lines were developed from each of the ten F1 individuals for genetic and mapping studies.

Three and a half thousand F2 plants derived from selfing the ten F1 POSH individuals were assessed in the field during the 2000 season for psr and agronomic traits. Eighty-one individuals were selected as being suitable for further development and F3, selfed seed collected from them, along with pods for RIT assessments and racemes to provide data on pod and raceme characteristics. **Analyses of these results, together with observations made as the plants were growing and at maturity, gave clear indications that it was...**

a) possible to select for greatly increased psr amongst the segregating F2 plants and

b) that this increased psr could be combined with good agronomic characteristics with interference from the deleterious traits commonly found when using synthetic napus in crossing programmes.

On the basis of observations and analyses of the data collected from the F2 population, the 81 selections were reduced to 30. However, in order to offset some of the problems associated with not being able to identify good psr until the plants are effectively dead with no flowers available for pollinations, a subset of five of the most promising were chosen to take forward in the first round of backcrossing to cv Apex; these were four POSH1-3 and one POSH1-2.

Seed of these five lines were raised to provide around 100 plants per line for backcrossing to cv Apex. In all, approximately 5000 backcross pollinations were made and, in addition, all the F3 plants were bagged to provide F4 seed. The F3 plants were also assessed for their agronomic characteristics and psr to facilitate the selection of the most promising backcrosses. Thus, four of the F3 individuals were designated as having the most suitable characteristics, and 120 backcross seeds of each of these was sown out, the resulting B1 plants selfed to produce B1S1 seed. Around 50 seed from 25 of the best plants in each line was then grown up to provide material for a field trial of the B1S1 generation to allow assessment of the levels of psr achieved during the selection and crossing procedures, combined with agronomic characteristics. Although rather late in the season, **this trial provided some very promising material for inclusion in the breeders' own programmes, combining good levels of psr with acceptable agronomy, without the deleterious traits the breeders were anxious to avoid. The trial also provided clear indications of the success of the project in managing to obtain high levels of psr amongst these potential breeding lines.**

Doubled haploid lines

Alongside the development and investigation of the F2 and backcross populations, microspore derived doubled haploid lines were produced from the six POSH1 and four POSH2 F1 individuals; these were to form mapping populations and to facilitate genetic studies of the psr trait. In all, 155 doubled haploid lines were produced for POSH1 and 64 for POSH2. All but three of these were available for a small-scale field trial carried out during 2001 to assess the levels of psr and other traits amongst the lines. Selfed seed was collected from each plant with, where required, some assistance from hand pollinations using salt solution to overcome incompatibility barriers often present in synthetics. A wide range of psr levels was observed across the lines, confirmed by the RIT assessments, from those lines which were very susceptible to those displaying very good resistance. Two of the lines, POSH1-2 and POSH1-3, contained the highest proportion of resistance, whilst POSH 1-1 was somewhat intermediate and the remainder similar to or worse than the moderately susceptible cv Apex. **These results revealed a similar picture to that observed amongst the F2 populations, and served to confirm that there was a sufficiently wide range of variability present to provide a suitable base for the mapping work which was to follow later in the project.**

No significant correlations were found between psr and the other, agronomic and pod related traits, providing some encouragement that it should be possible to select for increased levels of psr independently of the other traits, most importantly avoiding the problems of inadvertently selecting for any deleterious traits along with increased levels of psr. These findings, backed up by similar evidence from other data produced and analysed during the course of the project, ensures that **the breeders will be able to select for good psr without jeopardising the all important agronomic traits which contribute to the elite genotypes they seek.**

Genetic mapping

The application of molecular marker technologies at Bayer CropScience in Gent and the John Innes Centre in Norwich during the course of the project has resulted in the identification of certain regions of the *Brassica napus* genome having significant influence over the pod shatter resistance trait. **Three independent QTL have been identified by the Bayer CropScience partners and five possible QTL by the JIC partners**, and other QTL affecting seed and agronomic traits have also been detected. These finding require further work to substantiate the extent of the effects but they do provide **strong and encouraging indications of the potential for the application of marker assisted breeding and selection in the introgression of pod shatter resistance in commercial breeding programmes.** The option for this approach is particularly valuable given the difficulties in assessing the trait, and the lack of any easily measured associated traits which can be used as predictors of high levels of resistance during the period prior to senescence.

Pod shatter resistance in the resynthesized *Brassica napus* line DK142

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SUMMARY

Resistance to pod shatter was studied within and between populations of the resynthesized *Brassica napus* line DK142, grown under glass or in the field, at sites in the UK and Belgium. All populations showed similar ranges of resistance that were greater than that of a commercial cultivar (Apex). The increase in range was at least three-fold greater than the range found in Apex. Only sowing time affected the descriptive statistics of shatter resistance of each line, with spring-sown populations more shatter susceptible than winter-sown populations. The partitioning of dry matter to individual tissues of the pod was different for the two lines, with dry matter biased to the seed in Apex and to the receptacle in the more resistant DK142. In DK142 and Apex, as well as F1 and F2 populations derived from crosses between DK142 × Apex, correlations for pod shatter resistance and mature pod characters were high, particularly the weight and length of the valves and septum. Shatter resistance increased in plants when pod numbers were reduced by the removal of whole racemes.

INTRODUCTION

Oilseed rape (*Brassica napus*) is a relatively undeveloped crop compared with cereals and pulses. It has many weed-like characteristics, including pods that open (shatter) easily when ripe. Although of benefit to a wild species, this trait is highly detrimental in a crop, with considerable amounts of seed lost prior to harvest during adverse weather conditions and on contact with the combine during harvesting. Losses are typically quoted as 10% (Kadkol *et al*. 1984), but estimated between 20% (Price *et al,* 1996) to as high as 50% if harvesting is delayed (Child & Evans 1989; MacLeod 1981).

The anatomy of the pod relating to the process of opening has been described by Picart and Morgan (1984) and Meakin and Roberts (1990). Oilseed rape pods consist of two valves, each containing seeds, on both sides of a false septum. The valves of fully mature pods separate along dehiscence zones (DZs) which are situated within sutures that extend from the junction of the pod with the pedicel to the junction with the shrivelled style (beak). Current husbandry aims to reduce shatter by swathing or by the use of desiccant sprays just prior to full pod maturity. Both techniques desiccate and kill cells in the DZ preventing completion of the active process of cell separation. However, precise treatment timing in relation to stage of crop development is difficult to assess and asynchronous pod development, which results in differences in the time of seed maturation can affect seed quality. Increased shatter resistance should reduce husbandry inputs, improve seed recovery and may also enhance seed quality. Moreover, less outlay will be needed to control volunteer plants in subsequent crops and seed losses outside the field boundary should be reduced providing an added ecological benefit.

To some extent, the amount of shatter is influenced by the canopy architecture. Pods that are held erect in the canopy are thought to be more protected than pods that are horizontally orientated. Matting together of flexible racemes during the later stages of seed filling is also thought to reduce shatter (Thompson & Hughes 1986). However, although the canopy structure might affect efficiency of seed recovery, individual components of the canopy architecture could not be associated significantly with the inherent susceptibility

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of pods to shatter when they were tested away from canopy influences. The results of Morgan *et al*. (1998) showed that determinants of raceme morphology, such as pod angles, raceme length, thickness and width correlated poorly with pod shatter. However there appeared to be a tendency for tall plants with thick stems to be more shatter resistant.

Although estimates of overall shatter status have been made in field crops of commercial cultivars, it is possible that variation in shatter values exists between plants within the populations. However, shatter susceptibility appears to be fairly uniform between individual plants within commercial cultivars and the range of variation, which has not yet been reported, may be quite small. It might be of advantage to breeders to identify individuals with higher than average shatter resistance in order to determine the architectural structure of the pod that is associated with difficulty of valve separation which results in reduced seed loss.

In contrast with the current situation in commercial cultivars, the variation in shatter resistance identified within the amphidiploid species *B. juncea* and in *B. carinata,* as well as in *B. oleracea* and *B. rapa,* the putative diploid ancestors of amphidiploid *B. napus,* appears to be much greater, and individuals with high shatter values are known to exist (Bowman 1984; Downey & Robbelen 1989). Synthetic lines of *B. napus* derived from interspecific crosses between *B. oleracea alboglabra* and *B. rapa chinensis* have been reported to contain a wide variation in shatter resistance (Morgan *et al.* 1998). Several individuals were found to have high shatter resistance values when measured by a standard test procedure. Genes for shatter resistance acted recessively and correlations with most pod and plant morphological characters were low, suggesting that it would be feasible to introgress the shatter resistant trait into commercial breeding lines (Morgan *et al.* 2000).

The work reported in the present paper aimed to identify within the shatter-resistant, synthetic line DK142 and a more shatter-susceptible commercial cultivar Apex (i) plant to plant variation in shatter values, (ii) the stability of the resistance trait of DK142 in different environments and (iii), the possible relationship of shatter status with pod characters associated with valve separation. Shatter resistance was also determined in populations derived from crosses between DK142 and the commercial cultivar.

MATERIALS AND METHODS

Plant material

The *B. napus* line DK142 is derived from a selfed, doubled haploid, microspore-cultured plant that was itself derived from a cross between the doubled haploid winter breeding line N-0-109 (CPB Twyford Ltd, Hertfordshire, UK) and a synthetic interspecific hybrid of wild *B. oleracea alboglabra* and *B. rapa chinensis* at the John Innes Centre (Morgan *et al.* 2000).

Seed of DK142 for sowing in 1999 was produced from self-pollinated second-generation DK142 plants derived from the original microspore plant. Seed was bulked up in 2000 from cuttings taken from the second-generation DK142 plants. The shatter-susceptible commercial cultivar Apex was used as a standard comparison in all experiments. DK142 was cross-pollinated with Apex in May 1999 in order to provide seed for sowing early in August for the production of F1 plants in a heated glasshouse. The F1 plants were selfed to produce seed in time for raising F2 plants in the field during normal outdoor seasonal conditions.

Assessment of pod shatter resistance

Pod shatter was assessed by a 'random impact test' (RIT). This is a reproducible and controlled means of assessing pod shatter and aims to mimic conditions in the crop canopy during agitation by weather or machinery (Bruce *et al.* 2002). As the lignocellulosic property of the valves makes them hygroscopic and their water content can affect shatter, pods were equilibrated for at least 3 days at 25°C and 50% RH in a controlled environment cabinet before testing. For the test, 20 undamaged pods per plant were placed together with six steel balls of 12.5-mm-diameter in a 20-cm-diameter cylindrical container. The container was then mechanically shaken at a frequency of 4.98 Hz and a stroke length of 51 mm for two 10 s periods, followed where required by one period each of 20, 40 and 80 s. The times were chosen to give equal intervals after log transformation. At the end of each period, pods were examined and classed as shattered if one or both of the valves had detached. Using GenStat (Lawes Agricultural Trust, IACR-Rothamsted), the transformed data was fitted to a linear model and the time (s) taken for 50% of the pods to shatter (RIT_{50}) estimated, using a common slope and separate intercept model in sets of data grouped by commonality.

Morphological characterization and shatter resistance amongst populations of DK142 and Apex

DK142 and Apex seed was sown at the Long Ashton Research Station in John Innes No. 1 potting compost in December, 1999. Seedlings were transferred to John Innes No. 2 compost in 15-cm-diameter pots before

vernalization at the four-leaf stage at 6 ^{o}C for 8 weeks. The plants were then set out in March in an unheated glasshouse in seven blocks, each of which contained 16 plants of DK142 and Apex.

Stem height, measured as the distance from soil level to the base of the terminal raceme, was recorded at weekly intervals after the start of stem elongation in early March. Flowering began in April. Pollination was enhanced by bees from hives situated within the glasshouse, and by hand pollinations. Approximately 5 weeks after the start of flowering only a few flowers on the lowest primary raceme and on secondary racemes remained open and all pods on the terminal and first primary racemes had extended to their final length. At this time, two plants per line were chosen from each of the seven blocks of plants for uniformity on the basis of set and vigour. On each plant (n=14), all but the terminal and first primary racemes were removed and vegetative regrowth and secondary flowers were removed at weekly intervals.

All pods were fully mature by the last week of July. Pods were collected from the terminal and first primary raceme of all plants and 20 pods were assessed for shatter resistance by RIT. The lengths of a further ten pods were recorded and the weight of each pod component weighed separately after separating the valves from the beak plus septum and the seed. The number of seeds in each pod was also recorded.

Shatter resistance in DK142 and Apex in different field environments.

Seeds of DK142 and Apex were sown at Long Ashton, Norwich (John Innes Research Centre) and Gent (Bayer BioScience N.V.) during the winter of 2000 and at Gent and Long Ashton in early spring of 2001. The aim was to determine the extent of variation in shatter susceptibility in populations of each line grown under different environmental conditions.

Sowing times in 2000 and subsequent glasshouse conditions were varied from site to site during November and December in order to bring plants that were grown in John Innes No. 2 compost in 7.5-cm-diameter pots, to the four-leaf stage during January. Plants were vernalized at ambient temperatures in a gauze house at Long Ashton, under polythene at Gent and in controlled environment rooms at 6 °C for eight weeks at Norwich during January and February 2001. Losses occurred during winter but approximately 100 DK142 and 50 Apex were available for planting outside prior to stem extension during March at all three sites. Plants at all sites were grown 0.5m apart in double rows at least 1m apart. At Long Ashton, DK142 was grown in two rows and Apex in a single row of plants. At Gent, blocks of DK142 and Apex were grown within a larger planting scheme. At Norwich, plants were grown in rows of repeated sequences of five plants of DK142 and five of Apex. Standard agronomic treatments were used at each site to prevent pests and disease throughout development. Flowering took place at all sites during May. All pods were fully ripe by early August but weather conditions delayed collection of pods for analysis until September.

In Gent and Long Ashton, second sowings of DK142 and Apex were made in February 2001. Seedlings were artificially vernalized at 6 °C for eight weeks. In Gent plants were transplanted in the field on 14th June. Plants were grown in rows in blocks of 50 plants. Flowering during July was followed by an extended period of growth before 53 plants of DK142 were fully mature and ready for collection in October. Only 12 Apex plants were mature at this time and the remainder subsequently failed to ripen. At Long Ashton, plants were transferred to John Innes No. 2 compost in 15-cm-diameter pots and set out 0.5m apart in rows 1m apart in a cold glasshouse on 9th May. Each line was grown in rows of nine plants that were randomized in thirteen blocks. Flowering during late June and early July was followed by a period of 9-10 weeks before the pods of all plants were fully ripe in October, when 116 plants of DK142 and 58 of Apex were collected for RIT analysis

Shatter resistance amongst populations derived from crosses between DK142 and Apex.

The glasshouse-grown F1 plants were infected by much fungal growth during the later stages of pod development and although seed production for the F2s was unaffected, the pods were not suitable for shatter analysis. Therefore, a cutting from each of 40 F1 plants and from Apex plants were rooted in a propagator. These plants developed rapidly under glass, flowered and produced fully mature pods in July that were harvested and used for RIT shatter analysis.

Seeds of Apex and F2 were sown at Norwich and Gent in December 1999. At Gent seedlings were transferred to the field where they were planted 0.5m apart, in rows that were at least 1m apart. Vernalization took place outside during January and February 2000 at ambient temperatures. At Norwich seedlings were pricked out into John Innes No. 2 compost, vernalized in controlled environment rooms at 6 °C for eight weeks and then planted 0.5m apart, in rows that were at least 1m apart. Pods were harvested in the field as soon as possible after ripening in Gent in August and in early October at Norwich. Pods were collected from the terminal and first primary raceme of all plants and 20 pods were assessed for shatter resistance by RIT.

The lengths of a further ten pods were recorded and the weight of each pod component weighed separately after separating the valves from the beak plus septum and the seed. The number of seeds in each pod was also recorded.

Statistical analysis
Analyses of variance were carried out using the statistical package GENSTAT 5 (Genstat 2000) to calculate standard errors of differences between means. Where there were more than two treatments, significant differences at $P<0.05$ were calculated in t-tests.

RESULTS
Range of shatter susceptibility and components of pod structure in DK142 and Apex plants grown in a glasshouse during 2000.

Large differences in shatter susceptibility were recorded between populations of Apex and DK142 (Fig. 1). Although minimum shatter susceptibility (RIT_{50}) values were less than 10s in both lines and the mode was identical when using 10s class intervals, the mean RIT_{50} for DK142 (29s) was nearly two-fold greater than for Apex (15s) and the maximum for DK142 (99s) was three-fold greater. Moreover, when class intervals of 5s were used the mode of DK142 was 5s larger than for Apex. The distribution for DK142 was skewed compared with the near normal distribution in Apex . The increased range in DK142 was accounted for by 86% of its values that were greater than the mean for Apex and 33% of the values greater than the maximum RIT_{50} value of 32s for Apex.

Relationship between stem growth and shatter susceptibility
Stem height of Apex measured during and at the completion of extension had statistically significant correlation with RIT_{50} values of mature pods suggesting plant vigour has a role in shatter resistance (Table 1). For Apex, the positive relationship was established as soon as stem elongation began in early March. In contrast, the correlation with stem height was not significant in DK142 until final heights had been established in the middle of April and then the correlation was weaker than for Apex.

Morphological differences in pod structure
The external appearance of mature pods from the two lines was distinctly different (Fig. 2). DK142 pods had thicker pedicels, pod walls and beaks than Apex. Pods of DK142 were 15% shorter than Apex and contained only half the number of seeds (Table 2). However, the valves were more than 25% heavier and the beak plus septum more than 50% heavier than Apex. Despite the heavier receptacle of DK142, the total pod weight was 23% lighter than in Apex, because its seed weight was 55% less. Indeed, the partitioning ratio of seed to receptacle in Apex was 1.7:1, whereas for DK142 the ratio was 0.6:1.

For Apex the weight of the entire pod, the weight and length of the valves and the weight of the beak plus septum correlated significantly with one another ($r = 0.65$-0.91, all significant at 0.1%). All these parameters correlated significantly and positively with RIT_{50} values (Table 2). The correlation of the variation in seed weight and RIT_{50} values was only just significant. Similarly for DK142, the weight of the valves correlated well with the weight of the beak and septum ($r = 0.56$, significant at 0.1%) and valve length ($r = 0.62$) and as with Apex, longer and heavier pods were more resistant ($r = 0.47$-0.60). However, seed weight did not correlate significantly with RIT_{50} values.

As final plant height and pod characters of both lines were positively correlated with resistance, the relationship between plant height and pod characters was investigated (Table 2). Taller plants of both lines had significantly heavier pods. For Apex, heavier pods were a result solely of heavier valves, whereas all components of pods from tall plants of DK142 were heavier and in contrast with Apex the number and weight of seed was greater.

Effects of removal by pruning of all but terminal and first lateral racemes on shatter susceptibility, pod structure and size.
In the sub-set of 14 plants that had been pruned 5 weeks after anthesis, mature pods were approximately 50% more resistant than on the unpruned, control plants in both DK142 and Apex (Table 3).

Although fractionally longer, pods from pruned Apex plants weighed 39% more than pods from unpruned plants. Pods from pruned DK142 plants were not longer than from unpruned plants but were also significantly heavier by 20%. In both lines, pruning increased the weight of the pod receptacle more than the

weight of the seed. In Apex, receptacle and seed weight were increased by 45 and 35%, whilst in DK142 the increases were 26 and 7%. In Apex the weight of seed was increased as a consequence of more seed (Table 3). The extra pod receptacle weight was mainly a result of thicker valve walls, which in pods from pruned plants were heavier per unit length by 39% in Apex and 22% in DK142. Septum weight per unit length was also heavier by 23% and 10% in pods from pruned Apex and DK142 respectively.

Variation in shatter susceptibility within field grown populations of DK142 and Apex: Site to site comparisons for populations planted in the field in March 2001('early-sown').

For each of the lines the range of shatter resistance values (RIT_{50}) was similar at all sites (Fig. 3). In populations of Apex, the distribution at all sites were similar with an overall mean of 18 ± 0.7s. The range of resistance was narrow (<10-40s) and was normally distributed about a modal class of 10-20s. The mean RIT values for DK142 were on average three times greater than RIT_{50} values for Apex and the range of shatter values was much wider (<10s to 169s) with the most frequent scores between 25-40s. The mean values were statistically similar (P=0.23) at Long Ashton (51 ± 3.6s) and Gent (47 ± 3.2s) but a few high scoring values recorded in samples collected at Norwich increased the mean at this site to 73s. The distributions were broadly similar at each site and was skewed compared with Apex (Fig. 3) with over 90% of RIT_{50} values from each of the three DK142 populations greater than the Apex mean and between 52 and 73% of RIT_{50} values greater than the Apex maximum.

Populations planted in June 2001 ('late-sown').

The range and mean shatter resistance values for the 'late-sown' field grown population of DK142 at Gent (mean = 28 ± 3.2s; range <10 – 130s) were similar to those for the 'late-sown' population grown at Long Ashton under glass (mean = 24 ± 1.5s; range <10 – 100s). However, the mean shatter resistance values were approximately half those for the 'early-sown' populations (P<0.001) and the ranges were narrower. Moreover, for both 'late-sown' DK142 populations the mode was lower by 20s. Indeed, the frequency class range for the mode was shifted into the range typically occupied by 'early-sown' Apex.

The only population of 'late-sown' Apex that could be harvested was that grown under glass at Long Ashton. The range of resistance was much narrower (<10-22s) than for 'early-sown' populations and was normally distributed about a modal class of 0-10s which was lower than 'early-sown' populations by 10s. The mean value of 8s was less than half those for the 'early-sown' populations (P<0.001).

Clearly sowing late, planting out in June and harvesting in September caused an increase in shatter susceptibility compared with populations sown early, planted out in March and harvested in July. However, as seen in comparisons of 'early-sown' populations there was little site-to-site variation in the 'late-sown' populations and within each sowing period mean values were consistently three times greater for DK142 than for Apex.

Shatter susceptibility and pod characters in derived lines

RIT_{50} values in all F1 plants grown from cuttings in a glasshouse (Table 4) were similar to those recorded for pods from cuttings of the parent line Apex (mean RIT_{50}, 13s; range <10 – 25s). Pod length, valve and beak and septum weight of F1 pods were all similar to those of Apex (*cf.* Table 2; data for pod parameters from Apex cuttings were similar to those from plants grown from seed) and there were significant and positive correlations of the length and weight of the components of the pod receptacle with RIT_{50}. As with both parents, the mean weight and number of seed per pod did not correlate strongly with RIT_{50} values and both were greater than for DK142 though less than Apex.

In the F2 populations grown in the field at Norwich and Gent in 2000, the overall mean RIT_{50} was 127% greater than for Apex (Table 5). Although minimum RIT_{50} values in the F2 populations and in Apex were less than 10s, the maximum for the F2s was almost ten times greater than for Apex.

The distribution of the F2 values was similar to the generally more resistant parent (DK142) in that the distribution was wide and extended from <10 to >100s (Fig. 4). Resistance values for 98% of the plants were found in the continuous part of the range which extended to 110s, whilst two percent of the plants (four plants) had much higher RIT scores measured at 173, 193, 300 and 323s.

Data for pod parameters for Apex were similar to those given in Table 2 and are not shown. In the F2 population the range of values for the pod receptacle characters spanned the combined range of the two parent lines (*cf.* Tables 2 and 5). On average, pods tended to be heavier than both parents and F1 pods, a result of the interaction of inheriting pods which on average were as long as those from the commercial parent, but which also had the heavier valve weight per unit length of the near-synthetic parent (mean valve weight per

unit length for Apex, 0.82; DK142, 1.27; F2, 1.29). Correlations of the resistance scores with the various parameters confirmed the linkage of the length and particularly the weight of the pod receptacle with resistance (Table 5). This was also seen in the F1 population where the amount of variation in resistance values and pod dimensions was very much smaller.

DISCUSSION

Comparisons of the shatter distribution patterns for each of the DK142 and Apex populations identified a lack of environmental influence on shatter resistance mediated through site to site variation. Different geographical locations, growing conditions and growing year had little or no effect and for populations of Apex, the distribution and mean of shatter resistance in pot-grown plants under glass in 2000 was almost identical to those recorded in field-grown populations of 2000 and 'early-sown' 2001. The time of sowing was the only factor that influenced shatter susceptibility within populations of both lines. In 2001, the three 'early-sown' populations of both DK142 and Apex had similar shatter statistics within a line, with the mean RIT_{50} score for each line higher and the range wider than populations sown 'late' in 2001. For DK142 the distribution and mean of shatter resistance in pot-grown plants under glass in 2000 at Long Ashton was more similar to those recorded in the 'late-sown' rather than the 'early-sown' field-grown populations of 2001. Pot-grown plants were from sowings in December, which was relatively late compared with normal sowing times at Long Ashton and appears to have influenced the shatter statistics of DK142, although not Apex, in a similar manner to sowing 'late' in the field.

Plants set out in the field in June from 'late' sowings would have accumulated lower assimilate levels prior to flowering compared with the 'early-sown' plants. Indeed, the average stem height of 'early-sown' field-grown DK142 plants at Long Ashton was 634 mm compared with 501 mm for 'late-sown' plants. As crop height is clearly associated with total biomass, reduced plant weights in 'late-sown' plants may be relevant to the reduced resistance of DK142 planted out in June. Time of sowing did not affect Apex plant height, although the stems were thinner in the 'late-sown' population (data not shown).

Within individual populations of DK142 and Apex, plant to plant differences in height also correlated with shatter resistance (Table 1), with taller, more vigorous plants producing longer, heavier and more shatter resistant pods (Table 2). This positive relationship between increasing stem height and shatter resistance has been previously reported by Morgan *et al*. (2000). For Apex, the positive relationship of resistance with stem height was clear from the first week of stem elongation and may have been determined even earlier, whilst for DK142 the relationship was significant only when growth had finished. For both lines, the weight of the receptacle was heavier from taller plants, but for Apex, plant height did not correlate with seed weight per pod nor seed number. Thus, the partitioning of dry matter between the pods and their contents in tall Apex plants was similar to that found in DK142. Partitioning of assimilates to seeds appears to have been determined early in pod development whilst partitioning to the rest of the pod depended on availability of resources throughout development. Thus shatter resistance values may also be influenced throughout pod development.

The very large difference in shatter resistance values of Apex and DK142 strongly indicates a genetic component, but the importance of stem height and, therefore, plant vigour and biomass is particularly relevant to agronomic management. Thus, once the introgression of the DK142 shatter resistant trait into breeding lines is accomplished successfully, appropriate husbandry requirements may also need to be reassessed as recoverable yields may be further augmented by improvements in the nutritional status of the crop. This may be achieved by increased inputs, but improved individual plant biomass can also result from reduced sowing density and would promote shatter resistance *via* the formation of heavier pod receptacles. Moreover, in contrast with Apex, the seed weight from pods from taller, more vigorous plants of DK142 was increased as a result of increased seed number, thus improved nutritional status may further amplify the yield.

The distribution pattern of RIT_{50} values in the F1 population was more similar to that found in Apex than to that of DK142 suggesting shatter resistance is a recessive trait, whereas the distribution pattern recorded in F2 populations was similar to that recorded for DK142. This confirms the heritability of this trait and suggests that the genes and characters associated with increased shatter resistance are present in the populations of both the synthetic and the derived F2 plants.

The most marked difference between pods from the two parents was the weight and size of the receptacle and although DK142 pods were much shorter, they were heavier than those of Apex. Within line variation of these characters in the parent, F1 and F2 populations was monitored to determine which particular parameters determined shatter resistance. In all populations, including those with all or the

majority of plants falling within a small range of resistance, dimensions of the pod receptacle correlated positively with shatter. Variation in valve and beak plus septum weight produced the most significant correlations, closely followed by valve length and thus septum length. As both parents can contribute positive characters i.e., long pods from Apex and pods with a high weight/unit length from DK142, the range of resistance in segregating populations might have expected to exceed that found in the parents. This was the case in four plants in the F2 population, which had RIT scores higher than the range seen in DK142. These four plants had pods longer than the average DK142 pod, with three as long or longer than the average Apex pod and in each case, valve weight per unit length (1.5-2.11) was close to or equalled the maximum values found in the DK142 population.

Although the receptacle weight of the DK142 pods was heavier than in the longer Apex pod, the seed weight was 50% lighter due to 50% fewer seeds and contrasted strongly with Apex where the weight distribution was clearly partitioned towards the contents rather than the receptacle. The ranges of the seed and receptacle weights in the F2 population was similar to the combined ranges of the two parents but on average, mean receptacle weight was more closely aligned to DK142 and seed weight to Apex despite seed number being midway between the two parents. Thus, compared with the two parent lines, the F2 population showed improved resistance compared with Apex and improved seed set over DK142. Indeed, seed weight per pod was only 10% less than for Apex and although this is agronomically undesirable, the positive correlations of seed number and seed weight with RIT_{50} values in the F2 population suggests that restoring normal fertility should be compatible with retaining the enhanced shatter resistance.

The parental line difference in seed number per pod did not appear to result from differences in ovule numbers, which were similar in both lines in response to self- or cross pollinations by hand (data not shown). Although pollen production was delayed and reduced in DK142, lower seed number in this line may be more connected with partial infertility inherited from its resynthesized parent. We have also noted that line DK142 has a tendency to set fewer pods. Reduced seed and/or pod set in this line may be responsible for increased partitioning to the receptacle that may be a causal factor helping to determine increases in shatter resistance. However, within line DK142 the variation in number and weight of seed per pod did not correlate significantly with resistance so pod numbers per plant may be more relevant.

It was not known whether the heavier pod of DK142 was a cause or consequence of lower pod density or whether it was determined genetically by factors other than fertility. Removal by pruning of lower racemes and the consequent reduction in pod numbers enabled a comparison to be made of the effects of pod density on shatter susceptibility. Pruning increased the weight of the remaining pods by nearly 40 and 20% for Apex and DK142 respectively, with the increase in weight biased towards the seed receptacle. The RIT_{50} values in pods from pruned plants were on average 50% more resistant than pods from unpruned plants. As pods from pruned Apex plants were still more susceptible than those from DK142 with a similar number of pods, the results support the view that increased shatter resistance in DK142 pods is genetically determined. However, pruning enhanced resistance in both lines, suggesting competition for resources within the plant also helps to determine the final level of resistance. The genetic and competitive influences on shatter status may have different mechanisms, the resolution of which requires anatomical and perhaps biochemical study.

In all populations, the weight of the septum and of the valves each correlated well with resistance. These two components of the pod are joined at a dehiscence zone (DZ) which is located in sutures that extend on each side and along the length of the two valves. The DZ consists of rows of two or three simple, thin-walled cells that are separated from the valve by heavily thickened cells and which join with the heavily-vascularized replum of the septum. During the later stages of pod senescence, approximately 7 weeks after anthesis the thin-walled cells separate. The valves separate when impacts with other pods or with the combine break residual adhesions. Heavy receptacles may cushion DZ cells from impact, although if this were so, a strong positive correlation with pod wall thickness and resistance would be expected. As yet there is no published evidence that this is the case and it may be more likely that the weight of these organs affects resistance because they determine the size of the DZ. Clearly, valve length determines the length of the DZ and in all populations this parameter correlated well with resistance. However, although DK142 was more resistant, average pod length was greater in Apex than in the DK142. Correlations between the valve and septum weights with RIT_{50} in the parent lines and the F2 populations were also high and the more resistant parent line produced pods with heavier valves and septum despite their shorter length. We believe that pods with a heavier receptacle have a wider and thus more resistant DZ and that in addition, the DZ of DK142 has different separating properties compared with Apex. Investigations of the differences in DZ size as well as its structure in relation to pod wall architecture are underway in our

laboratory.

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Table 1. *Stem heights (mm) during and at the end of extension of DK142 and Apex grown under glass in 2000 and correlation (r) values of the variation in height with shatter resistance measured by a Random Impact Test (RIT_{50}). DK142, n=108; Apex, n=134*

	Min	**Max**	**Mean ± S.E.**	**r (RIT_{50})**
DK142				
One week	12	110	40 ± 1.3	0.11
Three weeks	47	323	141 ± 4.5	0.06
Final stem height	255	1000	636 ± 13.3	0.31**
Apex				
One week	0	15	1 ± 0.3	0.21*
Three weeks	0	134	28 ± 2.2	0.39***
Final stem height	33	845	434 ± 17.1	0.48***

, **, *,= r-values significant at 5, 1 and 0.1%.*

Table 2. *Descriptive statistics of mature pods of DK142 and Apex grown under glass in 2000 and correlation (r) values of the variation in pod parameters with final plant height and shatter resistance measured by a Random Impact Test (RIT_{50}). Values in parentheses are weight of organ as percentage of the total pod weight.*

	Line	Min	Max	Mean	S.E.D. (179 D.F.)	r (RIT_{50})	r (height)
Weight per pod (mg)							
Whole pod	DK142	86	232	143 (100)		0.52^{***}	0.41^{***}
	Apex	122	288	185 (100)	4.2	0.43^{***}	0.39^{***}
Valves	DK142	35	114	68 (47)		0.60^{***}	0.34^{***}
	Apex	30	90	53 (29)	1.9	0.49^{***}	0.61^{***}
Beak + septum	DK142	14	41	24 (17)		0.56^{***}	0.26^{**}
	Apex	10	27	16 (9)	0.6	0.50^{***}	0.13
Seed	DK142	6	96	52 (36)		0.15	0.31^{***}
	Apex	69	172	116 (63)	2.5	0.20^{*}	0.09
Number of seed per pod	DK142	2	18	11		0.13	0.35^{***}
	Apex	12	29	23	0.5	0.16	-0.13
Pod length (mm)	DK142	35	67	52		0.47^{***}	0.37^{***}
	Apex	50	69	61	0.7	0.31^{**}	0.26^{**}

*, **, ***, *r-values significant at 5, 1 and 0.1%.*

Table 3. *Effect of removal of all but the terminal and first lateral raceme on components of pod structure and shatter resistance measured by a Random Impact Test (RIT_{50}). Plants were grown under glass in 2000. Values in parentheses are weight of organ as percentage of the total pod weight*

	Apex		S.E.D.	DK142		S.E.
	Unpruned	Pruned	(86 D.F.)	Unpruned	Pruned	(86 D.F.)
RIT_{50}	14	22	1.5	27	41	3.7
Mature pods						
Weight per pod (mg)	176 (100)	245 (100)	5.7	141 (100)	168 (100)	8.1
Valves	50 (28)	75 (30)	2.6	66 (47)	85 (51)	4.0
Beak + septum	16 (9)	21 (9)	0.7	23 (16)	27 (16)	1.6
Seed	110 (63)	149 (61)	3.8	52 (37)	56 (34)	4.9
No. of seed per pod	23	26	0.9	11	11	1.0
Pod length (mm)	61	66	0.9	51	54	1.7

Table 4. *Characteristics of fully mature pods from F1 plants (n = 40) and correlation (r) values of shatter resistance measured by a Random Impact Test (RIT_{50}) with the variation in pod parameters. Values in parentheses are weight of organ as percentage of the total pod weight.*

	Min	**Max**	**Mean ± S.E.**	**r (RIT_{50})**
RIT_{50}	<10	36	16.9 ± 0.8	-
Weight per pod (mg)				
Valves	29	64	50 ± 1.3 (38)	0.45***
Septum	10	16	14 ± 0.2 (10)	0.40***
Seed	43	94	67 ± 2.2 (51)	0.18
No. of seed per pod	9	22	15 ± 0.5	0.04
Pod length (mm)	44	120	62 ± 1.9	0.21*

*, **, ***, *r-values significant at 5, 1 and 0.1%*

Table 5. *Characteristics of fully mature pods of F2* (n=197) *plants grown in the field at Norwich and Gent in 2000 (combined data) and correlation (r) values of shatter resistance measured by a Random Impact Test (RIT_{50}) with the variation in F2 pod parameters. Shatter resistance values for Apex plants (n =93) grown in the field are also presented as a comparison. Values in parentheses are weight of organ as percentage of the total pod weight.*

	Min	**Max**	**Mean ± S.E.**	**r (RIT_{50})**
RIT_{50} for F2	<10	324	41 ± 2.7	-
RIT_{50} for Apex	<10	35	18 ± 0.6	-
Weight per pod (mg) F2				
Whole pod			204 ± 4.5 (100)	
Valves	30	138	78 ± 1.6 (38)	0.58***
Beak + Septum	10	54	26 ± 0.6 (12)	0.50***
Seed	40	158	100 ± 2.6 (49)	0.29**
No. of seed per pod	6	27	17 ± 0.5	0.20**
Pod length (mm)	36	86	61 ± 0.6	0.26**

*, **, ***, *r-values significant at 5, 1 and 0.1%.*

Captions to figures

Fig. 1. Frequency distributions of shatter resistance measured by Random Impact Test (RIT_{50}) of Apex (closed squares) and DK142 (open squares) populations grown under glass in 2000.

Fig. 2. Typical pods from Apex (left) and DK142 (right)

Fig. 3. Frequency distributions of shatter resistance measured by Random Impact Test (RIT_{50}) of Apex (closed symbols) and DK142 (open symbols) populations grown during 2001 from 'early'- and 'late'-sowings at Long Ashton (squares), Norwich (circles) and Gent (triangles). Note the increased susceptibility of 'late'-sown populations.

Fig. 4. Frequency distributions of shatter resistance measured by Random Impact Test (RIT_{50}) of Apex (closed symbols) and F2 (open symbols) populations grown in the field in 2000.

Figure 1.

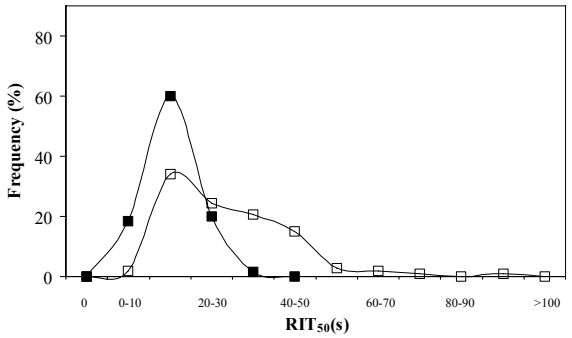


Figure 2.

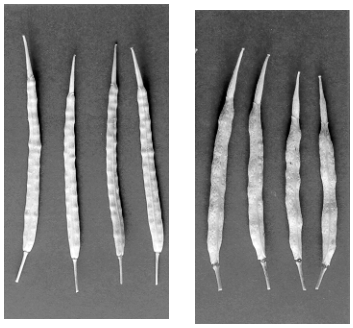


Figure 3.

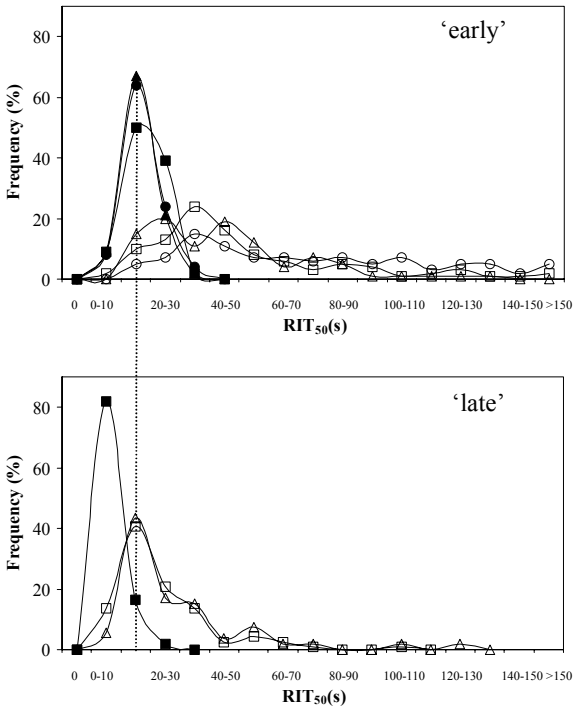
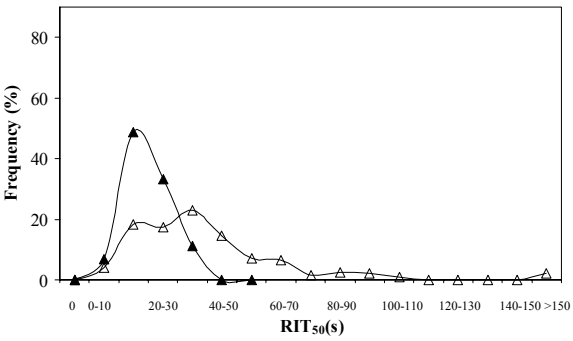


Figure 4



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Increased resistance to pod shatter is associated with changes in the vascular structure in pods of a resynthesized *Brassica napus* line.

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Abstract

The architecture of the pod wall and dehiscence zone (DZ) was studied in populations of a resynthesized, shatter resistant, oilseed rape line, DK142 and the commercial cultivar Apex. The dimensions of the pod wall and its component tissues were significantly larger in DK142. However, the variation in the pod architecture of Apex, DK142 and F2 populations derived from crosses of the two lines was found to have little or no role in pod shatter. In contrast, variation in the dimensions of the DZ characters correlated strongly and positively with shatter resistance. The size of the main vascular bundle (MVBV) of DK142 as it exited the valve and joined the vascular tissue of the replum was on average 60% larger than in Apex, the DZ was 40% wider and there was a high preponderance of vascular tissue other than the MVBV. The variation in the size of the MVBV accounted for much of the variation in shatter resistance of all populations, including shatter susceptible Apex. The DZ width was also found to be important in explaining the limited range of shatter values in Apex, but in populations of DK142 and F2, where the amount of vascular intrusion into the DZ was much greater, the variation in DZ width was not important. The importance of the vascular tissue to shatter resistance was further highlighted by a novel microfracture test (MFT). By contrast, no significant difference between DK142 and Apex in the ease of separation of the thin-walled DZ cells was detected using the MFT.

Key words: *Brassica napus*, cell separation, dehiscence zone, pod shatter, vascular tissue.

Introduction

Fully mature pods of oilseed rape (*Brassica napus* L.) are extremely sensitive to opening resulting in seed loss (pod shatter). This can take place prior to harvest due to disturbance of the canopy by wind or during harvesting as the combine machinery moves through the crop. Typical losses vary between 8-12% of the potential yield (Kadkol *et al.,* 1984) but reductions of up to 50% were estimated by MacLoed (1981) in seasons when weather conditions were poor prior to and during harvest. Reduction in the sensitivity of pods to opening would increase the proportion of the yield recovered by the combine and thereby improve production efficiency.

Little variation in shatter susceptibility was thought to be present in current commercial breeding lines (Bowman, 1984) but assessments by breeders of susceptibility to pod shatter between lines had to rely mainly upon visual observations of the crop in the field or upon hand tests of pods. However, a test procedure has been devised that exposes pods to random impacts in a similar manner to those that occur in the crop canopy during harvest (Bruce *et al*., 2002). This random impact test (RIT) enables the rapid comparison of susceptibility to shatter in samples of fully mature pods from individual plants. For example,

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Abbreviations: DZ, dehiscence zone; MFT, microfracture test; MVBV, main vascular bundle of the valve; MVBR, main vascular bundle of the replum; SEM, scanning electron microscopy.

in a population of 100 plants of the cultivar Jet Neuf subjected to the RIT, individual values ranged from 20% to 80% of the pods per plant opened after testing (Child and Huttly, 1999).

Sources of resistance to pod shatter have been sought amongst related species. More resistance is apparently present amongst some lines of *B. juncea, B. carinata* and *B. nigra* as well as amongst the putative parents of *B. napus* (*B. oleracea* and *B. rapa*) (Kirk and Hurlstone, 1983). Attempts to introgress shatter resistance traits from related species into *B. napus* can be complicated by linkage with unwanted characters. However, synthesis of new *B. napus* lines derived from interspecific crosses between *B. oleracea alboglabra* and *B. rapa chinensis* has provided a range of useful variation amongst which increased shatter resistance has been identified (Morgan *et al*., 1998). In contrast with the current situation in commercial cultivars, several individuals were found to have high shatter resistance values when measured using the RIT procedure. One of these high shatter resistant lines, DK142, showed similar ranges of resistance whether grown under glass or in the field, at sites in the UK and Belgium and the range was at least three-fold greater than the values found in the commercial cultivar Apex (Summers *et al.,* 2003).

Fully mature pods of *B. napus*, consist of two valves which are separated by sutures that extend on each side and along the whole length of the pod. Summers *et al*., (2003) found that although correlations were high between valve length and RIT in DK142 and Apex, the average length was greater in Apex than in the DK142. This suggested that increasing shatter resistance was more likely to be associated with differences in the pod wall and/or the dehiscence zone (DZ) structure rather than the overall pod size. The structure and development of the DZ in shatter susceptible cultivars has been described by Picart and Morgan (1984) and Meakin and Roberts (1990*a*).

In fully developed pods, the DZ consists of a layer of two-three, undifferentiated, thin-walled cells which separate the heavily lignified cells of the pericarp edge from the replum which consists of thickened schlerenchyma and is heavily vascularised. Degradation of the thin-walled cells is followed by their separation at about seven - eight weeks after anthesis (WAA). The process coincides with pod wall senescence and significant changes in enzyme and hormone activity (Meakin and Roberts, 1990*b*; Chauvaux *et al.,* 1997; Child *et al.,* 1998). Dissociation of thin-walled DZ cells initiates the process of valve separation but alone does not cause the detachment that results in seed loss. Separation of the valve from the replum takes place only after impact with other pods which severs the vascular connections between the valves and the replum.

Child and Huttly (1999) examined the surface of detached valves of the commercial *B.napus* cultivars Jet Neuf and Fido with a scanning electron microscope. They estimated that the large vascular traces at the pedicel end and the small, discrete traces that were widely spaced throughout the rest of the DZ surface accounted for a significant proportion of the total separation surface in some plants. It was not known whether these structural variations were genetic in origin or whether they were environmentally influenced. In this paper we show how the structure and dimensions of the DZ differ in DK142 from those in Apex and describe how these differences contribute quantitatively towards susceptibility to valve separation.

Materials and methods

Plant Material

Plants of Apex and DK142 were grown under glasshouse conditions at Long Ashton Research Station (LARS), Bristol, UK in 2000. F2 plants were grown in the field at the John Innes Centre (JIC), Norwich UK during 2000. Full details of the growing conditions and the methods of collection of fully mature pods for shatter assessment by RIT have been described elsewhere (Summers et al*., 2003).*

Light Microscopy

Flowers were labelled at anthesis and five weeks later, pods that were fully developed but still green, were collected for microscopy. Fresh material was prepared for determination of pod wall dimensions as follows. Transverse sections approximately ten microns in thickness were cut by hand using a single-sided razor blade, from the middle of a fresh pod from each of 100

plants per parent line and from each of 107 F2 plants. Sections were cut through the middle of a seed so that the tissue slice included the thinnest part of the wall adjacent to a seed and the opposite, thicker wall on the non-seeded side. Each section was immersed for one minute in an aqueous 0.01% solution of crystal violet that stained the thickened cells in the endocarp and the vascular tissue violet. All other parts of the wall remained unstained. The sections were surface-lit and measurements of the pod wall were made using a low power dissection microscope. The minimum width of the pod was measured in the line of the septum between the base of each suture. The maximum pod width and the width of the thickened and unthicked portions of the wall of each loculus were measured at 90° to the septum. The number of rows of cells on both sides of the pod were also recorded in the thickened portion.

The structure of the DZ and the arrangement of the vascular tissue in the replum were determined in embedded material. Transverse slices of pods approximately one-mm-thick were fixed in a mixture of 4% paraformaldehyde and 5% gluteraldehyde for one hour under reduced vacuum. The material was washed in water and dehydrated in a series of solutions of ethanol of increasing strength over a period of two days, and then infiltrated and embedded in LR white acrylic resin (TAAB laboratories, Berkshire, UK) over a period of three days. Transverse sections two microns in thickness were cut on an Ultracut microtome (Reichert-Jung, Vienna, Austria), mounted on slides and stained with an aqueous mixture of 0.1% methylene blue and 0.1% Azure A. Unthickened cell walls stained dark blue. Parallel studies with phloroglucinol identified lignified cells which stained turquoise when treated with the methylene blue/Azure A mixture. The sections were examined using a Leica DMRB microscope.

Scanning Electron Microscopy

Mature pods from each of 100 plants of DK142 and Apex and from 38 F2 plants were collected after the entire plant had senesced and dried out. The F2 plants were selected from 107 plants to represent the full range of shatter values based on RIT results. Gentle pressure was applied by hand to up to eight pods from each plant to separate the valves along the suture line. The basal end of one of the valves was cut from each pod, approximately 7-8 mm in length, mounted on aluminium stubs and coated under vacuum with a film of fine-grain gold using a Polaron E5000 sputter coater. The separated surface of the pericarp edge was examined under a Phillips 505 scanning electron microscope (SEM). Images of standard fields of view for each valve were recorded at a magnification of ×300 after calibration with an SIRA SEM scanner (Agar Scientific, Stansted, UK) and dimensions of the vascular tissue and the DZ were measured. The width of the DZ was measured at its widest point at the pedicel end, which included the fractured surface of the main vascular bundle of the valve (MVBV) and along the edge of the valve, where the loculus was at its full width. The width and breadth of the MVBV was also measured and the records used to calculate its approximate area. The amount of vascular tissue, excluding the MVBV was estimated as a percentage of the DZ total area. For F2 material, only the area of small, vascular bundles adjacent to the MVBV was estimated as a percentage. When present, the length of longitudinal bundles lying along the edge of the DZ in the same field of view was also measured.

Fracture analysis.

An analysis to establish the contribution to resistance by the vascular tissue of the DZ was carried out using pods from selected plants. A micro-fracture test (MFT) was developed at the Silsoe Research Institute to establish the contribution of the MVBV to the amount of energy needed to separate the valve from the replum. Pods from six plants of DK142 and six of Apex were selected to represent the full range of shatter resistance for each population. The range of RIT values for 50% of pods to open for Apex was <10-32 s and for DK142 was <10-99 s. Thus plants with similar resistance values were included from both lines as well as DK142 plants with RIT values which were greater than the maximum value of 32 s for Apex.

Pod wall tissue was excised with a small, circular saw (Minicraft, Spennymooor, Co. Durham, UK) to isolate areas for testing that were approximately one mm in length. These

isolated areas consisted of the septum and valve, between which the DZ remained intact and were prepared either from the pedicel end of the valve including the MVBV or from the middle of the pod about half way between the pedicel and the beak and did not contain any vascular tissue.

With sections at the pedicel end of the valve, cuts were made at approximately 45° to the longitudinal axis of the pod to isolate the tip from the remainder of the valve (Fig. 1A). The pedicel was held vertically in a small chuck and the pod beyond the test zone was cut away using the saw. An L-shaped, steel device in which the horizontal projection was 0.5 mm, was mounted on a force transducer of range 2 N, and positioned so that it contacted just the valve tip (Fig. 1B). The device was raised by a Universal Test Machine (Model DN-10, Davenport-Nene, Wellingborough, Northants, UK) until fracture of the specimen occurred. After fracturing, the broken section of valve remained in place because a 'peg' of vascular tissue projecting from the valve part of the test piece remained located in the corresponding hole in the replum. This attachment allowed energy stored elastically to be recovered.

For sections from the middle of the pod, the MFT required the firm bonding of pods to the base of the test machine *via* micro translation stages (Fig. 1C). The L-shaped steel device was manoeuvred under the isolated section and then raised at a rate of 1 mm min^{-1}, while force and vertical movement were recorded (Fig. 1D). When a break was detected by a sudden drop in force, the vertical movement was reversed to lower the section of pod wall until the force reached zero. This action allowed the energy stored by elastic deflection of the test section to be recovered and quantified. The peak force needed to break the sample and the net energy expended were then calculated.

After completion of the MFT, the fracture surface of the piece of valve removed during testing was coated with gold and the total area of the DZ and that of the MVBV was determined during examination under the SEM.

Results

Morphology of pods in DK142 and Apex

Although the number of ovules was similar in DK142 and Apex at anthesis, the number that developed into mature seeds in DK142 was approximately 50% lower than in Apex. Anthers did not dehisce until two days after anthesis when they opened only at the distal end. Pollen production was much less than in Apex although cross pollinations by hand with DK142 pollen resulted in Apex pods with full compliments of seeds whilst selfing of DK142 or cross pollination with Apex resulted in approximately half the number of ovules developing to seeds. It was concluded that although DK142 pollen was fertile, successful pollination may have been impeded either by reduced ovule fertility or by changes in receptacle structure which obstructed pollen tube growth.

Individual pods were fully mature in Apex between 9 and 10 WAA, but DK142 pods took up to 4 weeks longer. The time taken for individual plants of DK142 to fully senesce did not correlate with shatter resistance. The length of fully developed DK142 pods varied from 35 to 65 mm between plants and on average were 15% shorter than those of Apex, in which pod lengths were more consistent. Pod shape was generally similar in both lines (Fig. 2) although in all DK142 plants, the valves were on average 29% wider when measured between the sutures along the septum and 18% wider at 90° to the septum (Table 1). The surface of the pod was smoother but less regular in width in DK142 because of the widely spaced seeds, which were approximately 50% fewer.

Pedicels were consistently thicker in DK142 than in Apex. In the pods of some DK142 plants, there was a distance of up to 10 mm between the valve and the swollen scar tissue of the sepals, petals and anthers located at the distal end of the pedicel (Fig. 2). The base of each valve was rounded in both lines but was blunt and obtuse in Apex and more tapered in DK142. The replum was also thick in DK142 causing each valve base to arch away from the replum forming a 'lip' at an angle of approximately 30°. In Apex, the valve base did not protrude and lay flat against the replum. Valve edge definition was clearer and the suture

wider in DK142 than in Apex. The beaks of DK142 were similar in length to those of Apex but were wider at the intersection with the valves.

In Apex, the outline of the main vascular bundle (MVBV) was clearly visible in the middle of each valve and along its length, smaller vascular traces joined it at irregular intervals. This is shown diagramatically in Fig. 2D. One or two longitudinal vascular bundles connected the small traces along the length of the valve and appeared to terminate near the junction of the valve with the pedicel close to the MVBV. Other vascular traces passed into the replum near the valve edge at regular intervals. All the vascular bundles were raised on the surface of the pods forming clear 'ribs' in Apex but in DK142 an apparently similar arrangement of vascular tissues was much more difficult to identify externally because of the thicker pod wall. When the valves were detached in fully mature pods the main vascular bundles of each replum (MVBR) to which seeds were attached by their funicle could be seen at the base of each suture. A papery false septum joined the two MVBRs and contained no vascular tissue.

Pericarp wall structure in fully developed pods

Tissue architecture and the extent of thickening in the pod wall were determined in fresh, hand-cut sections taken from the middle of the pod (Fig. 3). Three WAA, when pods had extended to their final length, thickening was present in the endocarp of DK142 but not in Apex. By five WAA, thickening was complete in both lines and significant differences in internal architecture were established. The total width of the pericarp and the width of thickened band of cells in the endocarp varied in relation to position in the pod. In both lines, endocarp cell wall thickening was greatest in the non-seeded areas and least on the seeded side of the pod (Fig. 3. A, B). In Apex, only a single row of endocarp cells was often present, although the average was 1.7 rows on the seeded side and 2.2 on the unseeded side of the pod. The thickened zone was on average 0.17 mm wide and was surrounded by the non-thickened zone 0.24 mm wide. In DK142, the thickened zone was 165% wider than Apex not only because it contained on average an extra row of cells but because the radial length of each cell was 67% greater. Up to 35 vascular traces were present in the mesocarp of both lines adjacent to the unthickened exocarp (Fig. 3). At maturity, after drying down and the shrinkage of the unthickened exocarp these traces were prominent on the surface of the pod in Apex, giving it the 'ribbed' appearance described above. In DK142, the thickened zones on both sides of the pod subsumed the vascular traces and after drying down, the surface of these pods was smooth and not ribbed.

Correlations between the dimensions of the pericarp wall and its components in green pods were subsequently determined with shatter resistance values in an RIT of sub sets of fully mature pods (Table 1). Overall, correlations with RIT values were low but were statistically significant for the pod diameter measured at 90° to the septum, for pod wall thickness and for radial cell length. The correlation of pod wall thickness with resistance was mediated in Apex through the variation in the width of the thickened zone, whereas in DK142 it was associated with the width of the non-thickened zone. There was no significant correlation with RIT for the number of rows of cells in the thickened zone in DK142 or Apex.

Vascular architecture in fully developed pods

The tissue embedded at five WAA was used to determine the architecture of the vascular system and in particular, it's spatial distribution at the valve edges. Just before the junction with the two valves the pedicel was swollen by scar tissue of the abscinded anthers, petals and shrivelled remains of the sepals. Just after this position the replum tissue consisted of an outer cortex composed of thickened collenchyma cells immediately below the epidermis and an inner layer of loose parenchyma cells. Groups of fibrous cells were associated with a ring of collateral vascular bundles (Fig. 4A, F). The fibrovascular tissue of the stele was arranged in two large groups of bundles that were separated from two opposite, single bundles by parenchymatous medullary rays that connected the cortex to the central medulla. The two separate, single bundles diverged from the stele into the cortex at the intersection with the base of the valve (Fig. 4B, G). These passed through the thin-walled cells of the DZ into the

valves forming connecting 'bridges' of vascular tissue (Fig. 4C, D, H) forming the main vascular bundle of each valve (MVBV). Later examination of mature valves under the SEM, confirmed that in Apex this vascular 'bridge' was formed at an angle of divergence 70 – 90° to the vascular tissue of the stele. In DK142, the angle of divergence was much less and when the distance between the scar tissue and the junction of the valve with the septum was greatest the angle was less than 20°.

After the divergence of the single bundles to the two MVBVs, the two remaining groups of bundles passed into opposite sides of the edge of the septum to form the main vascular bundles of the replum (MVBR). Vascular bundles could be seen passing between the replum and the valve for a distance of 5 mm (Fig. 4E). The number and angle of these bundles differed between Apex and DK142. This was shown later and with greater clarity in the SEM examination (see below). In Apex, the valves widened rapidly at approximately 5-7 mm from their base after which the loculi were wide enough to contain seed. However, in DK142, the valves remained narrow in some plants for up to 1 cm. From this point onwards sections showed that the vascular architecture in the DZ, replum and MVBR was similar in both lines. The two replums were joined by the thin, papery septum composed of unthickened, loosely associated cells. No vascular tissue was present in the septum, which forms a partition in the pod cavity between the two loculi

In transverse sections of embedded tissue taken from the middle of the pod five WAA (Fig. 3C, D) it was clear that there were no differences between DK142 and Apex in the structure of the DZ at the junction with the suture and around the replum. The number of thin-walled cells that were later to separate was the same in both lines. Vascular traces were occasionally found adjacent to the pericarp edge.

Fracture surfaces of detached pods

Examination of the fracture surfaces of detached valves under the SEM showed that the DZ was wider at the junction with the pedicel than along each of the valve edges bordering the loculi. In DK142 (Fig. 5A), the pedicel end of the DZ was 91% wider than in Apex (Fig. 5B) and the range of values in individual plants was 365% greater (Table 2). In the DZ bordering the loculi, the average width was greater by 40% and the range greater by 45%. In both lines, there was a high level of correlation between DZ width at the pedicel end of the valve with the variation in shatter resistance measured by RIT, but the correlation where the loculus was at its full width was only significant for Apex.

In both DK142 and Apex, the dissociated DZ cells had usually separated along the line of their middle lamellas and had dispersed leaving an imprint of their walls on the pericarp edge that they had lined. At the pedicel end, the imprint suggested that the cleanly separated and now dispersed cells were arranged indeterminately forming an irregular surface (Fig. 5C, E, F). However, in DK142 at the junction with the pedicel, the valve arched away from the rest of the pod at an angle of about 30° and the thin-walled cells were orientated at a more oblique angle to the surface of the DZ than in Apex. Along the edge of the loculus, the remaining imprints of both lines were flat in appearance and were orientated along the surface of the DZ e.g. DK142 in Fig. 5D. Remnants of torn walls could clearly be seen at the junction of the inner edge of the DZ with the lining of the pericarp wall. In Apex there were one or two rows but in DK142 four or five rows of torn cells could be seen.

Cracked surfaces of vascular bundles were clearly visible and sometimes associated with tearing of adjacent thin-walled cells. The MVBV was the largest of the vascular bundles, situated towards the inner edge of the DZ at the pedicel end in both lines. In Apex (Fig. 5B, F) it fractured cleanly, exposing a single, discrete, transverse section. In DK142, the MVBV was usually much wider, lay at a shallower angle and was apparently composed of several vascular tissue groups each of which fractured separately, often at different levels, resulting in a multi-faceted stump (Fig. 5A, B, C). All MVBV size parameters were significantly larger in DK142 than in Apex (Table 2). However, the size of the MVBV correlated positively with resistance and with a similarly high degree of statistical significance in both DK142 and Apex, despite the lower between plant variation in populations of Apex.

In DK142, longitudinal bundles arose from the MVBV and ran parallel with the fractured surface of the DZ (Fig. 5A). In the DZ bordering the loculus in mid-pod sections, longitudinal vascular bundles smaller than those near the MVBV were found at irregular intervals (see also Fig. 3C). These were also seen in Apex, but vascular bundles in longitudinal orientation at the fracture surface adjacent to the MVBV were never seen. In contrast, the longitudinal bundles of the Apex valve arose from the main vascular tissue of the replum and crossed the DZ surface close to the MVBV at an angle of approximately 90° (Fig. 5B). Thus, the longitudinal bundles of Apex valves have minimal contact with the DZ and do not contribute to the size of the MVBV, but in DK142 these bundles have maximal DZ intrusion whilst also contributing to the size of the MVBV.

Fracture analysis

The peak force and energy required in the MFT to separate the isolated piece of pod wall from the replum at the pedicel end varied considerably within and between lines Apex (Table 3). On average, line DK142 required 118% more force and 161% more energy than Apex. For Apex, the variation in the peak force and total fracture energy required to separate the small pieces of pod did not correlate significantly with the shatter resistance values measured on whole pods, but for DK142 the correlations were highly significant.

After carrying out the MFT, the detached valve pieces were examined under the SEM (Fig. 5E, F), and the area of the fractured surface of the MVBV and the non-vascular DZ cells calculated. The total DZ area in DK142 was larger than in Apex because the DZ was wider and the MVBV of both lines comprised a similarly small percentage of the total test area (approximately 9.7% for Apex and 11.3% DK142). However, as found with previous SEM analyses of the DZ (Table 2), the average MVBV was larger than in Apex.

For Apex, the variation in the area of the MVBV and particularly the much larger area of the non-vascular DZ tissue correlated significantly with the peak force and total fracture energy required to break open these small sections, but neither area related significantly with shatter resistance of entire pods. For DK142, despite the fact that the non-vascular tissue was larger than the MVBV, only the variation in the area of MVBV correlated significantly with peak force and total fracture energy and this area also correlated significantly with shatter resistance of whole pods.

Isolated sections taken from the middle of the pod were also microfracture tested, examined under the SEM (Fig. 5D) and the few containing vascular tissue discounted in the analyses. The DZ in Apex was 0.23 mm wide but in DK142 was 48% wider (Table 4). A peak force of 0.25 N and fracture energy of 18 μJ was required to break open Apex sections, whereas corresponding values in DK142 were 132% and 72% greater. However, the energy per unit area was similar for both lines (P=0.67), but peak force per unit area was 50% greater for DK142 compared with Apex (P< 0.05).

Transmissibility of DZ characters and resistance into F2 populations

The range of values for pod wall dimensions in the F2 population was closest to that seen in the resistant parent (DK142), but the mean for most parameters was intermediate between the two parent lines (cf. Table 1 and 5). The range in shatter resistance measured by RIT was similar to DK142 and values ranged from <10 to 324 s. However, when the variation in the range of the pod parameters was correlated with shatter resistance the only relationship that was significant was with the width of the non-thickened zone and this was at a low level of significance.

Sections of the valves from a sub-sample of the F2 plants whose shatter resistance spanned the full range were examined under the SEM. The mean and range of values found for the length, width and area of the MVBV were more similar to those found in DK142 than Apex (cf. Tables 2 and 6). Correlations of the variation in these parameters with RIT values confirmed the importance of the size and in particular the width of the MVBV to shatter resistance. Longitudinal vascular bundles situated near the MVBV and parallel to the DZ were present in many of the valves as in DK142. The length of these bundles in the F2 valves correlated with a high degree of significance with shatter resistance, but in contrast, there was

no significant correlation with the area occupied by bundles that had fractured in transverse section.

Discussion

The range of shatter values recorded in populations of DK142 is wide and continuous indicating polygenic variation. This contrasts with Apex which shows a much narrower range of shatter susceptibility (Summers *et al.,* 2003). Although some correlations of this variation in shatter susceptibility with that measured in several pod wall characters were significant (Table 1), the level of significance was low. Moreover, in the F2s only the width of the non-thickened zone 5WAA showed any significant correlation with shatter values recorded in this population. This correlation was also at a low level of significance, which is not surprising, because during senescence the cells within this zone collapse forming only a thin layer around the thickened zone, contributing little to the overall pod dimension. Thus, we conclude that pod wall dimensions play only a small part, if any, in shatter resistance. A similar conclusion was found in a comparable study with sesame seed capsules. As with oilseed rape, sesame seed loss before harvest is a major economic problem, but none of the anatomical features of the capsule correlated significantly with seed retention (Day, 2000).

Josefsson (1968) believed that lignification in the pod at the junction of the valves with the replum in *B. napus* was relevant to shatter susceptibility. It provided a firm edge to the valve against which the thin walled cells formed the line of weakness. Genes expressed in the valve margins have been identified in *Arabidopsis* e.g. SHATTERPROOF1 and SHATTERPROOF2 appear to regulate lignification of the valve margins (Liljegren *et al.,* 2000). Spence *et al.,* (1996) have suggested that tension can develop in the pericarp wall of *B. napus* and *B. juncea* and in *Arabidopsis* during desiccation as a result of differential thickening and that this contributes towards the separation of the thin walled cells in the DZ. However, the significant increase in the amount of thickening in the endocarp of DK142 compared with Apex did not correlate with RIT and it seems unlikely that changes in the pod wall architecture play an important role in determining shatter susceptibility.

In contrast, overall pod dimensions of mature oilseed rape pods, particularly the length and weight of the septum and valves, have been shown to correlate with a high degree of significance with shatter resistance (Summers *et al.,* 2003). Thus, we concentrated on the role of the DZ, the tissue that interfaces between these organs and which is ultimately responsible for determining how readily the valves separate from the septum. For all DZ characters, the range of values found in Apex was narrower and the mean values lower than the wider ranges and higher means found in populations of DK142. This distribution of variation was similar to that found in the RIT scores (Summers *et al.,* 2003) and the variation of many of the DZ features correlated significantly with the variation in shatter scores (Table 2). In particular, the highly significant correlations of MVBV size with RIT values in both parent lines and subsequently in the F2 populations indicated and then established that the size of this tissue plays a key role in shatter resistance. The new micro-fracture technique applied to sections in which the MVBV with some surrounding undifferentiated cells was isolated from the majority of the DZ, confirmed this relationship in the resistant parent. In both lines, the MVBV comprised approximately 10-11% of the DZ area of these sections and each section was no more than 2% of the entire pod. Despite this, the force and energy required to break open small sections of DK142 correlated with high significance with shatter resistance of whole pods. Moreover, peak force and energy required in these sections correlated significantly with the size of the MVBV and not the area of non-vascular tissue. For Apex, the correlation of shatter resistance with MVBV size was not significant and the peak force and energy required in these sections correlated with greater significance with the area of non-vascular tissue. Tests further down the pod confirmed the relationships that had previously been seen in the SEM examination (Table 3) between DZ width and shatter resistance. For the susceptible parent (Apex) with its small MVBV and narrow range in shatter susceptibility the relationship was highly significant, but in contrast, the variation in DZ width in populations of DK142 was not important to its wide variation in shatter susceptibility. Thus for resistant

pods, the size of the large MVBV determines the upper limit to pod strength and influences from the variation in DZ width are less important. Weak pods, not only of Apex but including weak pods of DK142 and F2, always had a small MVBV and because of this, the width of the DZ was able to contribute proportionately more to pod strength.

Resistant DK142 and F2 pods are stronger than susceptible pods because of the larger MVBV but it is possible that other structural changes contribute to the overall increase in shatter resistance. These arise from: a) the angle at which the MVBV and adjacent vascular tissue pass through the DZ into the replum and, b) the longitudinal bundles between the undifferentiated cells and the replum which present additional disruption to the separation of the valve. Apex had very little additional vascular tissue surrounding the MVBV and that which was present was transversely orientated and the amount did not correlate significantly with shatter resistance (Table 2). In contrast, longitudinally orientated bundles as well as transverse bundles commonly surrounded the MVBV of DK142. For this line the amount of vascular tissue surrounding the MVBV correlated with high degree of significance with shatter resistance. Moreover, in F2 populations where a more detailed analysis of the surrounding vascular tissue was performed, correlations indicated that only the longitudinal bundles were important in determining resistance. If longitudinal bundles are stronger because of their direction, it is likely that the more longitudinally orientated MVBV of DK142 is likely to be stronger per unit area than that of Apex.

Views of the separated valve surface under the SEM suggested that tearing in more rows of cells might have taken place in DK142 than in Apex and the microfracture test on side sections confirmed that sections of DK142 required 50% more force per unit area to separate them. This was because the DZ of DK142 was 40-50% wider. However, a similar amount of energy per unit area was required to separate the undifferentiated, simple cells in both lines as the extra number of torn rows of cells in DK142 was in proportion to its wider DZ compared with Apex. Thus, although the wider DZ of DK142 gives additional resistance, between line differences in cell separations are not important. Furthermore, no reduction in cellulase activity has been found in the DZ of DK142 (Osborne, private communication). Thus, in the absence of a significant difference in hydrolase activity it is not surprising that differences in the pattern of cell separation were not large in the fully mature, separated valves of DK142 and Apex when examined under the SEM. This contrasts with loss of shedding competence in a non-abscinding mutant of *Lupinus angustifolius* cv Danja associated with loss of cellulase function (Henderson *et al*., 2001). It seems clear therefore, that the increase in shatter resistance in DK142 is due to the size of its DZ and DZ components particularly the size of its vascular tissue.

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Summers JE, Bruce DM, Vancanneyt G, Redig P, Werner CP, Morgan C, Child RD. 2003. Pod shatter resistance in the resynthesized *Brassica napus* line DK142. *Journal of Agricultural Science* (in press)

Table 1. *Dimensions of pod and pod wall components measured in transverse hand-sections of DK142 and Apex 5WAA and correlation (r) of the plant to plant variation with shatter resistance measured by RIT. Plants were grown under glass at LARS (n=100 for both lines).*

		Range	Mean	P-value	r (RIT)
Pod wall thickness (mm)	DK 142	0.50-1.33	0.71		0.23*
	Apex	0.30-0.63	0.40	<0.001	0.25*
Thickened zone (mm)	DK 142	0.25-1.00	0.45		0.14
	Apex	0.05-0.38	0.17	<0.001	0.28**
Non-thickened zone (mm)	DK 142	0.15-0.48	0.27		0.21*
	Apex	0.15-0.38	0.24	<0.001	0.00
Mean radial cell length in thickened zone (mm)	DK 142	0.07-0.30	0.15		0.29**
	Apex	0.00-0.25	0.09	<0.001	0.23*
Pod width along septum (mm)	DK 142	2.80-4.30	3.47		0.05
	Apex	2.00-3.30	2.69	<0.001	0.04
Pod width 90 ° to septum (mm)	DK 142	4.20-6.80	5.24		0.19*
	Apex	3.20-5.50	4.44	<0.001	0.23*
RIT	DK142	<10-99	29		-
	Apex	<10-32	15	<0.001	-

*, **, ***, *r-values significant at 5, 1 and 0.1%.*

Table 2. *SEM analysis of DZ characters of valve sections from the pedicel end of DK142 and Apex. Plant to plant variation was correlated (r) with shatter resistance measured by RIT. Plants were grown under glass at LARS (n=100 for both lines).*

		Range	Mean	P-value	r(RIT)
DZ width bordering loculi (mm)	DK 142	0.20-0.42	0.35		-0.02
	Apex	0.22-0.29	0.25	<0.001	0.44***
DZ width at pedicel end (mm)	DK 142	0.34-2.19	0.67		0.28**
	Apex	0.25-0.60	0.35	<0.001	0.44***
MVBVarea (mm^2)	DK 142	0.005-0.096	0.038		0.41***
	Apex	0.009-0.044	0.023	<0.001	0.50***
MVBVwidth (μm)	DK 142	156-532	282		0.37***
	Apex	147-344	231	<0.001	0.48***
MVBVlength (μm)	DK 142	33-262	166		0.33***
	Apex	74-205	127	<0.001	0.39***
Vascular tissue excluding MVBV (% of DZ in section)	DK 142	20-100	79		0.33***
	Apex	10-70	30	<0.001	0.12
RIT	DK 142	<10-99	29		-
	Apex	<10-32	15	<0.001	-

*, **, ***, *r-values significant at 5, 1 and 0.1%.*

Table 3. *Microfracture analysis of isolated sections at the pedicel end of the pod including the MVBV. Plant to plant variation in Apex and DK142 was correlated (r) with shatter resistance measured by RIT. Isolated sections were excised from five pods from each of six plants per line. Apex, n=26; DK142, n=27.*

		Range	Mean	P-value	Correlation (r) with		
					RIT	Peak Force	Fracture Energy
Peak Force (N)	DK142	0.29-3.00	1.33		0.64***	1.00	0.97***
	Apex	0.15-1.00	0.61	<0.001	0.02	1.00	0.75***
Fracture Energy (μJ)	DK142	9-265	81		0.69***	0.97***	1.00
	Apex	5-90	31	<0.001	-0.08	0.75***	1.00
Area occupied by MVBV(mm^2)	DK142	0.03-0.09	0.06		0.79***	0.84***	0.87***
	Apex	0.02-0.05	0.04	<0.001	0.29	0.41*	0.48*
Area of non-vascular DZ (mm^2)	DK142	0.07-0.70	0.47		0.18	0.19	0.23
	Apex	0.16-0.59	0.37	<0.05	0.27	0.69***	0.66***

*, **, ***, *r-values significant at 5, 1 and 0.1%.*

Table 4. *Microfracture analysis of isolated mid-pod sections of DK142 and Apex. Plant to plant variation in Apex and DK142 was correlated (r) with shatter resistance measured by RIT. One section from three pods from six plants was tested. Apex, n=15; DK142, n=18.*

		Range	Mean	P-value
Peak Force (N)	DK142	0.18-1.16	0.58	
	Apex	0.10-0.59	0.25	<0.001
Fracture Energy (μJ)	DK142	3-95	31	
	Apex	2-68	18	0.12
DZ width (mm)	DK142	0.27-0.44	0.34	
	Apex	0.12-0.30	0.23	<0.001
DZ area (mm^2)	DK142	0.21-0.35	0.28	
	Apex	0.11-0.25	0.19	<0.001
Peak Force per unit area (N mm^{-2})	DK142	0.7-3.8	2.1	
	Apex	0.5-3.0	1.4	<0.05
Fracture Energy per unit area (μJ mm^{-2})	DK142	11-295	104	
	Apex	12-338	91	0.67

Table 5. *Dimensions of pod and pod wall components measured in transverse hand-sections of F2 pods 5WAA and correlation (r) of the plant to plant variation with shatter resistance measured by RIT. Plants were grown in the field at JIC (n=107).*

	Range	Mean	r (RIT)
Pod wall thickness (mm)	0.43-1.00	0.62	0.14
Thickened zone (mm)	0.08-0.63	0.31	-0.01
Non-thickened zone (mm)	0.15-0.60	0.32	0.22*
Mean radial cell length in thickened zone (mm)	0.07-0.33	0.13	-0.04
Pod width along septum (mm)	2.10-5.70	3.56	-0.02
Pod width 90° to septum (mm)	3.60-9.40	5.51	0.14
RIT	<10-324	56	-

, **, *, r-values significant at 5, 1 and 0.1%.*

Table 6. *SEM analysis of DZ characters of valve sections from the pedicel end of F2 pods. Plant to plant variation was correlated (r) with shatter resistance measured by RIT. Plants were grown in the field at JIC (n=38).*

	Range	Mean	r(RIT)
DZ width bordering loculi (mm)	0.27-0.49	0.38	0.11
DZ width at pedicel end (mm)	0.35-0.86	0.56	0.19
MVBV area (mm^2)	0.007-0.081	0.037	0.70***
MVBV width (μm)	139-492	287	0.68***
MVBV length (μm)	49-451	164	0.32*
Length of longitudinal bundles (μm)	0-417	146	0.63***
Vascular tissue excluding MVBV (% of DZ in section)	2.5-17.5	7.5	-0.16
RIT	<10-324	69	1.00

, **, *, r-values significant at 5, 1 and 0.1%.*

Legends for figures.

Fig. 1. Procedures for the micro-fracture test (MFT). A. Pedicel end of valve with portions removed to isolate section containing the MVBV (cf Figure 5 E and F). B. L-shaped, steel device inserted under the edge of the valve (mounted vertically) ready for raising with Universal test machine. C. Isolated sections prepared from the middle of the pod (mounted horizontally) for testing. D. L-shaped steel device inserted beneath valve and raised to separate the DZ.

Fig. 2. Fully mature pods of DK142 have dense walls and are more irregularly seeded (B, C) than Apex (A). Replum extension between the end of the valve and the scar tissue is frequently seen in DK142 (C). The distribution of the vascular bundles in a single valve is shown diagrammatically in D.

Fig. 3. Transverse, hand-cut sections show the wide, thickened endocarp consisting of elongated cells arranged radially and engulfing the vascular traces in DK142 (A). In section B (Apex), the endocarp is narrower, composed of rounded cells and is separate from the vascular traces. Pod wall measurements (←→) were taken adjacent to the main vascular bundle (MVBV). Details of the DZ and replum are shown in semi-thin, transverse sections (C, D). The shape and arrangement of tissues in the DZ and replum were similar in DK142 (C) and Apex (D). The thin-walled cells of the DZ can be seen between the edge of the heavily stained pericarp (p) and the replum (r). A longitudinal vascular bundle lies between the pericarp edge and the replum in DK142 (C, arrowed).

Fig. 4. Semi-thin sections of the valve end of the pod of DK142 (A – E) and Apex (F – I) 5WAA. The main vascular bundle (MVBV) is indicated by an arrow. The two MVBVs arise at opposite sides of the replum from the vascular tissue of the stele (st) between the scar tissue (sc) of the petals and anthers and the base of the valve (A, F). Each migrates towards the pericarp edge through the cortex (c) (B, G) and passes through the DZ (C, H) into the valve (D, E, I). In Apex, the MVBV crosses the DZ in a different orientation to DK142 (cf. D, H). Smaller vascular traces adjacent to the MVBV can also be seen crossing the DZ of DK142 (E). At 7WAA, the DZ cells have separated but the valve remains attached by the vascular tissue at its inner edge (I). lo, loculus; v, valve.

Fig. 5. Detached sections of fully mature pods of DK142 (A, C, E) and Apex (B, F) from the pedicel end of the valve viewed under the SEM. Sections D-F are pieces from the MFT, with D (DK142) isolated from the middle of the pod and showing thin-walled cells in the pericarp edge arranged in parallel rows. Longitudinal vascular tissue (lvt) lies at the edge of the DZ in DK142 (A) but not in Apex (B). In DK142, the main vascular bundle (MVBV) is large, fractures irregularly and protrudes from the DZ (A, C, E). In Apex, the MVBV is small and fractures along one plane. The ends of smaller bundles close to the fractured end of the MVBV can be seen in A, B and C. Fractured remnants of some of the thin-walled cells of the DZ can be seen between the inner edge of the MVBV and the pod wall in DK142 (E) and Apex (F). pw, pod wall.

Figure 1

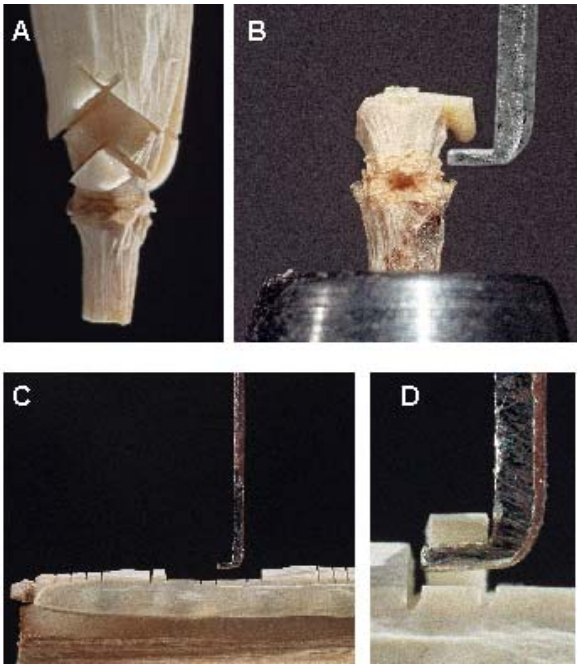


Figure 2

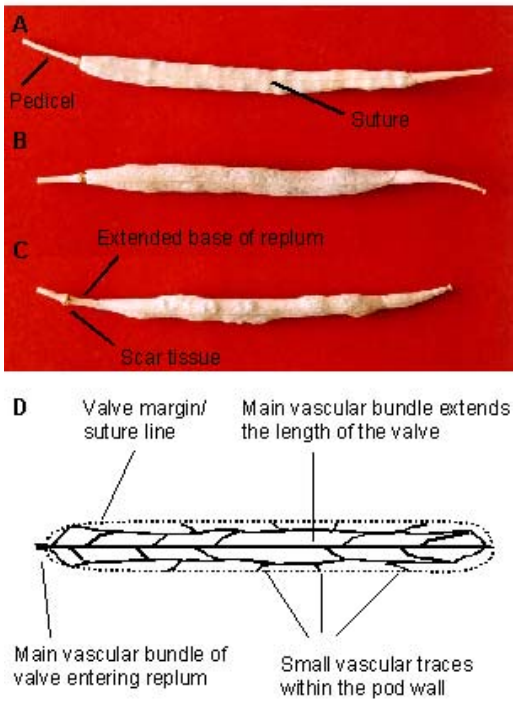


Figure 3

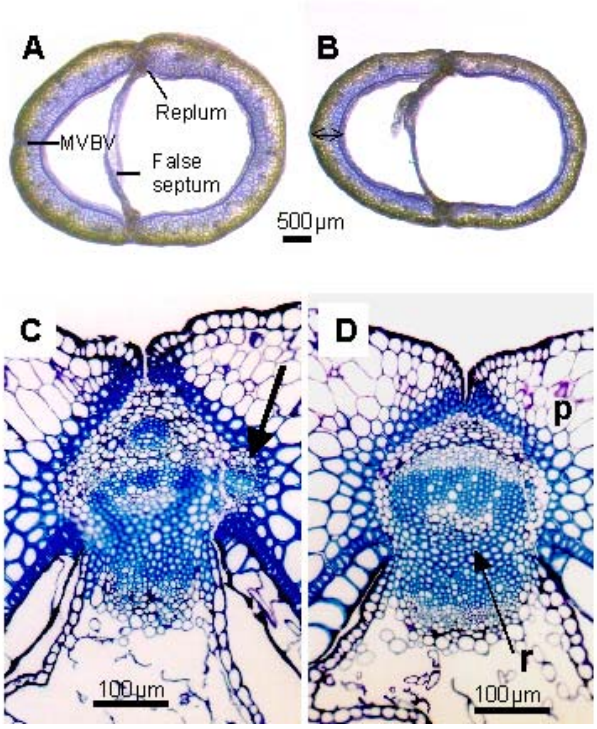


Figure 4

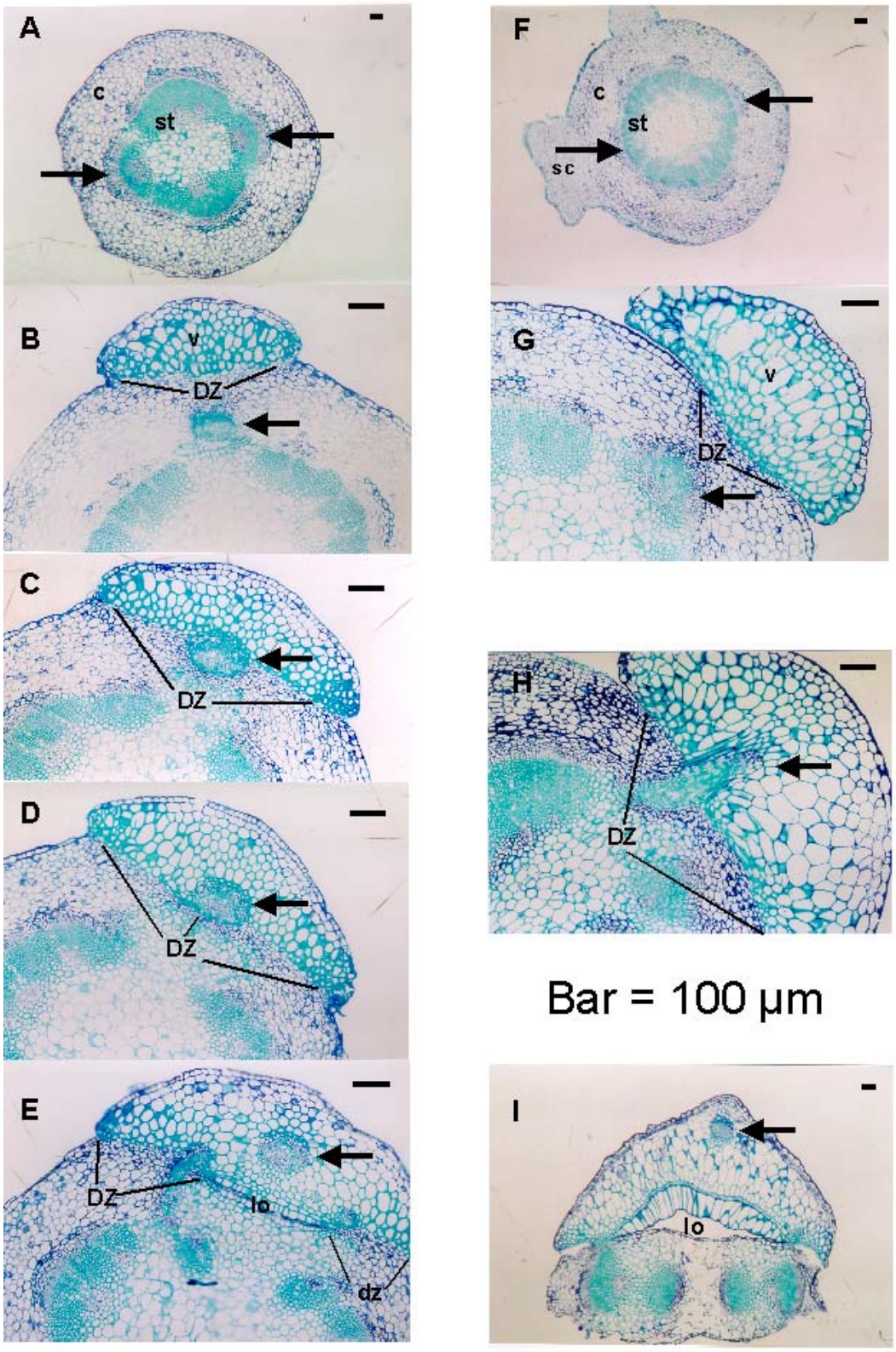
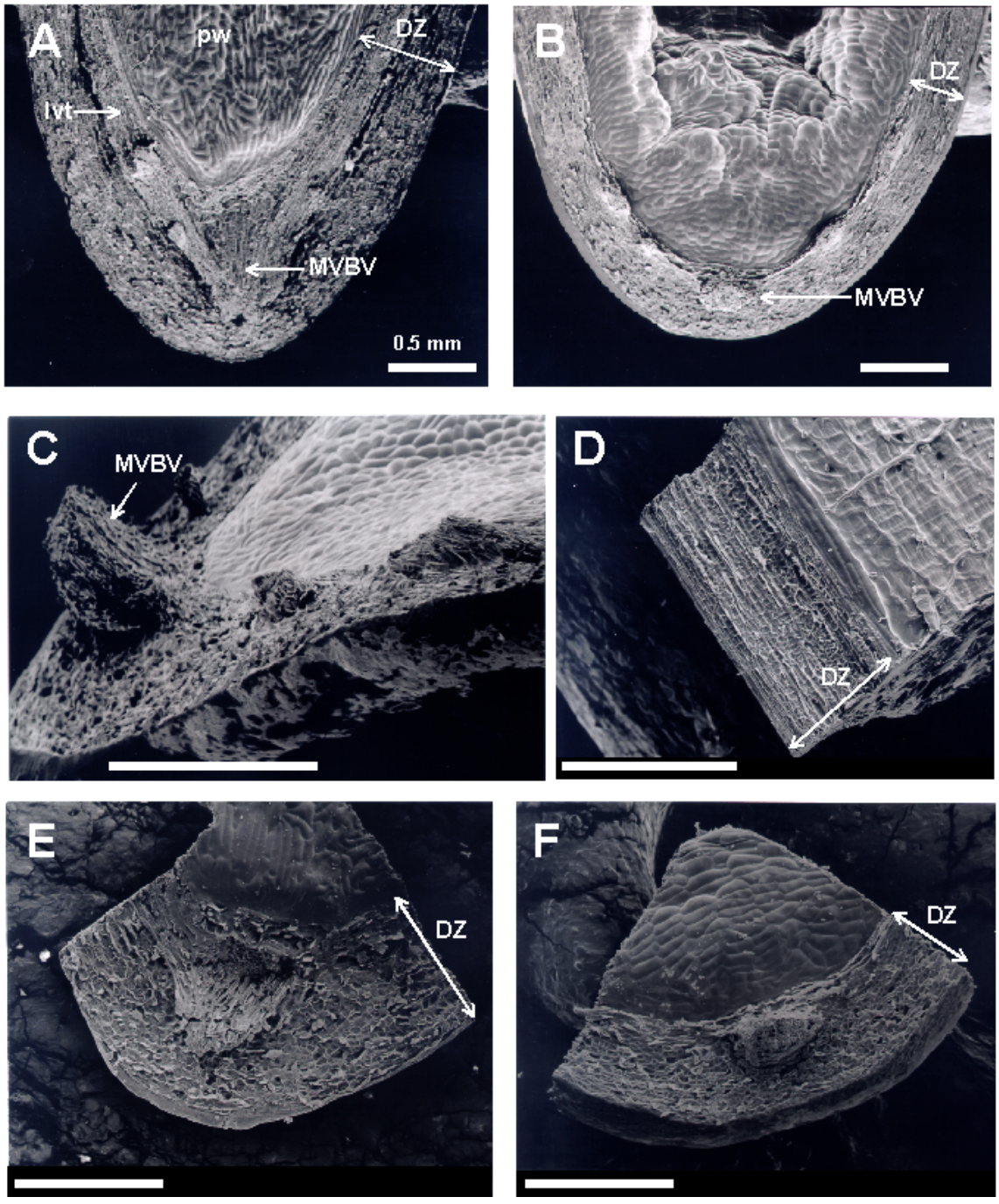


Figure 5



Introgression of pod shatter resistance into commercial breeding material: I F2 segregation

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INTRODUCTION

Pod Shatter Resistant Parents

From amongst populations of synthetic *Brassica napus* produced at the John Innes Centre, two synthetic doubled haploid lines were identified as having particularly high levels of pod shatter resistance (psr) when grown as part of a larger experiment designed to assess variation on this novel material. These lines, coded DK142 and DK129, were subsequently included in experiments to investigate the genetic control of the psr trait (described in the paper by Morgan et al, 2000, included above), and contributed to the SAPPIO Link programme for use in this project. Their derivation and use in this project is illustrated in Fig 1 where it can be seen that the two psr synthetic doubled haploid lines were used as female parents in crosses with cv Apex to produce F1 progenies POSH1 and POSH2

Commercial Parent

The cultivar Apex was chosen as the pollen donor parent by the two commercial partners as the most suitable elite line for this purpose. The cultivar was, at the time of the choice, one of the most widely grown, with excellent yield and agronomic characteristics, and possessed one of the best levels of psr amongst the cultivars available at the time. However, to put this level of resistance into perspective in relation to that found in DK142, DK129 and other cultivars, when psr is estimated on a scale of 0 (= highly susceptible to shattering) to 5 (= highly resistance to shattering), Apex scores around 2 whereas both synthetic lines score around 4. Other cultivars assessed at the time scored less than Apex, and most were between 0 and 1 on the scale.

Fertility issues in synthetics

However, whilst the choice of Apex as a parent for the crosses to be used in the project provided a strong, vigorous, highly fertile and productive plants, the same cannot be said for the two synthetics, and some serious problems were encountered in the early stages of working with these lines. Both seemed reluctant to set selfed seed, although pollen production in both lines appeared to be relatively good. A characteristic of DK142 which might contribute to its reluctance to set selfed seed is that it possess very long styles, placing the stigmatic surface well above the anthers and possibly reducing the opportunity for pollen to be deposited in self pollination. However, despite some (relatively simplistic) investigations into the likely causes of these difficulties, no particular reason for their low fertility was found, other than the general one related to their synthetic origin. It is not unusual for synthetic material, produced through the recreation of the *Brassica napus* genome from crosses between its diploid parents, to exhibit "symptoms" of low fertility, caused, it is presumed, by problems at the genome level in accommodating and integrating the two diploid sets of chromosomes into a single genome, to function as a diploid (amphidiploid).

A further, unrelated suggestion was considered, based on an early assumption that the increased levels of psr observed in DK142 might be due to decreased activity of the cellulase enzyme, polygalacturonase (PG). Work elsewhere (Peterson *et.al.*, 1996) was suggesting that at least part of the cause of increased resistance to shattering in some cases is due to the failure of this enzyme to function in its role of breaking down the cells in the dehiscence zone, the ring of cells around the pod which, when sufficiently weakened by the enzyme action, allows the pod to split open (shatter). PG is also responsible for a similar mechanism which

enables the anthers to split open and release their pollen (and in the abscision of leaves and seeds from their funicle, but all are controlled by specific temporal and spatial promoters to ensure that the cell degradation process occurs at the appropriate time and place in the plant's development). Thus, it was deemed, the failure of PG to function properly might be the cause of resistance in DK142 and, to some extent, interfere the normal function of the anthers, thus reducing fertility. This idea was also supported by discovering that DK142 produced increased numbers of pods and seeds when pollen from other sources was used in hand pollinations or wind pollination in the field, compared to seed production from bagged plants left to self pollinate.

However, despite extensive investigations into these problems, no definite conclusions were reached about the activity of PG in DK142 and it was decided that the main reason for its poor fertility lay in its synthetic origin, although lowered PG activity may be the cause of increased resistance in some of the doubled haploid lines derived from the crosses of the two synthetic lines with cv Apex. Furthermore, if the rare and potentially valuable improved psr discovered within these synthetic lines was to be exploited commercially, any problems encountered would have to be overcome. Thus the POSH1 and POSH2 crosses formed the backbone of this project designed to provide the commercial partners with the psr trait in a genetic background best suited to their own needs.

MATERIALS AND METHODS

Two synthetic lines of *Brassica napus L,* DK142 and DK129, each derived from a selfed, doubled haploid, microspore-cultured plant produced from a cross between a doubled haploid winter breeding line and a synthetic interspecific hybrid of wild *B. oleracea alboglabra* and *B. rapa chinensis,* were used as parental lines contributing pod shatter resistance. At the outset of the programme little was known concerning the agronomic and breeding potential of the material other than the identification of very significant levels of resistance to pod shatter. These two parental lines were each crossed as female parents to the winter oilseed rape cultivar Apex. The F1s were coded:

POSH_1 = DK_142 x APEX
POSH_2 = DK_129 x APEX

F1 plants from 6 POSH_1 and 4 POSH_2 individuals were self pollinated in the glasshouse to produce several grammes of seed from each F1 individual. Owing to the unstable nature of the near-synthetics DK142 and DK 129 these F1 and derived F2 families were treated as separate populations.

CPBT testing of F2 progenies

and F2 individuals were transplanted to the field in 2000. Positive selection was imposed for a return to the desirable agronomic traits shown by Apex. Flower type and pollen production was noted to be abnormal and was assessed. Selected individuals were bagged and further selected during pod development. Final selection in the field used a manual impact test (MIT) (**REF**) to identify the more shatter resistant individual at harvest. Pods were collected for shatter resistance testing using the random impact test (RIT) method (**REF**).

JIC testing of F2 progenies

The F2 seed, produced by self pollinating the six POSH1 and four POSH2 F1 plants, was grown out in a field experiment at JIC during the 2000 season. **[how many???]** plants of each of the ten POSH families were grown in two separate blocks, in staggered double rows 1.5m apart and 0.5m between plants within rows. Seed was germinated in the glasshouse and when the seedlings were six weeks old, they were vernalised for six weeks in 8 hour days at 6^0C, grown on in the glasshouse for a further four weeks before being planted in their final positions in the field at the end of April 2000. The usual plant husbandary conditions were applied.

A list of the assessments made on the plants during their flowering period in June and July is given in Table 3. In view of the relatively large numbers of individual plants involved in this assessment, traits were assessed by scoring according to the categories given on the list in the Table, rather than the more usual procedure at JIC of making detailed measurements to provide metrical data for statistical analysis. Although the analysis of such categorical data may be rather less precise than metrical data, the objective here in assessing these segregating F2 families was to identify levels of psr in the mature plants and retain a "data picture" of the agronomy of the plants in their flowering and maturity periods. This information was also used to investigate the presence of any significant relationships which might exist between easily observable, agronomic traits and any increased levels of psr; such a relationship would be invaluable in helping to identify individuals with potentially good psr at an early stage and help to overcome the problem of timing of selection of psr in relation to flowering (see below). Categorical assessments were deemed sufficient for these purposes and were considerably easier and quicker to perform on the relatively large numbers being dealt with than the more detailed but very time consuming and labour intensive metrical measurements.

At the end of the growing season, when the plants were fully mature, senesced and dry, all were assessed for psr using the Manual Impact Test (MIT). This simply involves handling the pods and giving them a score for the plant on the scale of 0 (= highly susceptible to shattering) to 5 (= highly resistant to shattering). From amongst the plants with good levels of resistance, with an MIT score of 4 or more, selections which combined good psr with commercially acceptable agronomic characteristics were identified, labelled and the plants retained. Open pollinated seed was collected from these plants as a backup since, although most of these selections had been bagged to guarantee self pollination, the open pollinated seed would provide a useful reserve of the genes producing these superior, selected individuals, should it be required at a later date. To increase the chance of collecting seed which had been produced as the result of self rather than open (cross) pollination, and thus increase its value to the breeding programme, pods from the centre of each plant were chosen. In addition, a single primary raceme was removed from the selected plants for detailed measurements to be made on pod characteristics, and at least 30 pods were taken from each selected individual for Random Impact Tests (RIT) to be carried out to determine a standardised evaluation of psr for these selected individuals, which would facilitate further selections from amongst this selected group.

Eighty on plants were selected in total from the F2 population. These came from the six POSH1 and four POSH2 lines as follows…

POSH Line	No. individuals selected
1.1	10
1.2	6
1.3	19
1.4	9
1.5	0
1.6	2
2.1	3
2.2	21 (but poor seed set)
2.3	11
2.4	0
	---
	81 F2 individuals selected in total

These 81 individuals were reduced further to 30 on the basis of the RIT scores and further consideration of the agronomic suitability of the F2 individuals; these 30 lines were to be taken forward to the next phase of the programme as the F3 generation for backcrossing to cv Apex.

RESULTS

CPBT testing of F2 progenies

The F2 populations grew well and flowered only about 10-15 days later than the conventional autumn sown oilseed rape in the same field. A total of 777 individuals (of the 3750 F2 plants) were selected at flowering time and bagged on the terminal and/or side branch. These were selected as acceptable plants of reasonable agronomic type. Considerable variation existed within this group of selected plants but it could be noted that in general the plants showed good vigour but were 'fleshy' and tended to be too vegetative. POSH 1 F2 families but not POSH 2 families were segregating for an abnormal reduced petal (narrower), with a severe petal fold and, most significantly, poor pollen production. The frequencies of this abberation is shown in Table 1. Normal flower anatomy was also associated with normal seeming pollen production.

At maturity all the bagged plants were scored for the manual impact test (MIT) and those scoring 6 and above (unless definitely very poor agronomically) were harvested. Both self and OP pods were kept. Notes were made on the general appearance and fertility. It was also observed that there were some plants showing a strong replumb at the base of the pod and others with average replumb but a series of attachments along the length of the pod giving a 'popper' effect. These differences were recorded with the general notes. Study of these notes shows that the POSH 2 shatter resistant plants all fall into the 'popper' category whereas most of the POSH 1 resistant plants were not 'popper' types. Table 2 summarises the numbers and results of the MIT assessments. Fig 2. shows the results of the random impact test conducted on pod samples collected from 19 F2 individuals plus 3 Apex samples as controls.

JIC testing of F2 Progenies

Data collected from the F2 plants grown in the field in 2000 for the traits listed in Table 3 were subjected to statistical analyses to determine the means and standard deviations, significance of the variation amongst and within lines, and correlations amongst the traits.

Means and standard deviations for the listed traits are presented in Table 4. For all but two of the traits, Flower Colour and Fasciation, the data were normally distributed and could, thus, be subjected to the usual parametric statistical analyses; the two exceptions were omitted from these analyses but note taken of the means and the data examined carefully for any indications of meaningful segregation ratios or patterns, but nothing sensible could be made of these.

Little variation between lines was observed for Flowering Time but the lines themselves, as expected for a segregating population, showed some differences in their variability, with the POSH2 lines generally having higher standard deviations than POSH1. It can also be seen that the POSH2 lines were segregating for Flower Colour, with genes for some paler colours than the usual yellow coming from the DK129 parent. POSH2 lines also differed in exhibiting extensive fasciation in some plants, although none was seen in any of the POSH1 plants. Otherwise, no striking differences were seen amongst the ten POSH lines for the morphological traits, with the means being very similar and the similar standard deviations an indication that the extent of segregation within the lines was approximately the same.

The range of MIT scores, the assessment of psr by handling the pods at full maturity and assigning a score between 0 and 5, implied that, on average, there was little improvement in

shatter resistance over Apex (which usually gave a score of around 2 on this scale) but some individuals within lines exhibited much improved values as can be seen for the results for some selected lines presented in Table 5. A more detailed assessment of psr amongst these F2 progenies was provided by the RIT carried out on the 81 selections made from amongst the F2 population, the results for which are given in Table 6. Most notable are the results for POSH1.2, POSH1,3, POSH1.4 and POSH2.2 which still had intact pods remaining after 80 seconds bombardment in the RIT machine. Notable, too, are the low scores for POSH1.1, POSH1.6, POSH2.1, POSH2.3 and cv Apex for which no intact pods remained after the 80 seconds period.

These findings were a significant milestone in the project - the expression of improved psr present in the DK parents, and on which the objectives of the project were founded, were being revealed amongst the segregating F2 progeny, demonstrating the feasibility of selecting for genetically determined psr in this way.

However, one of the major concerns of the commercial partners was the likely presence of deleterious traits such as the fasciation prevalent in the POSH2 F2 population. The existence of tangible psr which could be selected and bred for would be of little value if it was irretrievably linked to some deleterious trait such as this. Some measure of these associations can be provided through statistical correlations, and results of these calculated amongst the traits scored on the F2 populations is given in Table 7. Here it can be seen that there are no values above +/-0.3 and most are closer to 0, giving a clear indication of the lack of associations amonst these traits. Notable are the low values of the correlations involving RIT, the standard assessment of psr in this project, and this was to be the case for all the correlation analyses carried out on other, similar data sets. Thus, it should be possible for the breeders to breed for improved psr without being seriously hampered by undesirable traits.

Results for some of the pod related characters assessed on a range of F2 individuals selected with improved psr and good agronomy are given in Table 5. The information presented here is not intended to be exhaustive but to give an indication of the range of values for some of the pod related traits, in comparison with Apex. Mostly, the values for the selected POSH plants compare favourably with those for Apex, differing most noticeably in the numbers of seeds per pod. These are redeemed somewhat, however, by the much higher RIT scores for the POSH selections - indeed, for this data set, whilst Apex failed to have any intact pods remaining after 40 seconds in the RIT machine, some of the better POSH selections had significant proportions of intact pods remaining after 100 seconds bombardment.

Although seed number per pod was somewhat lacking in the POSH selections compared to Apex, mean seed weight was as good and usually higher for the POSH selections, but no estimates of total yield were made for this material. Thus, for the most part, the results indicated that the traits measured fell within range of those for the Apex control, with the possible exception of seed number per pod.

Returning to the RIT assessments, we can see from the three graphs in Fig.1 the extent of the improvement in psr of two POSH selections over Apex. RIT results for Apex are given in the top graph where there is a clear and sharp decline in the curve from 20 intact pods at the start of the test to 0 at 40 seconds. In the second graph, for POSH selection 997, the pods take longer to break (shatter) on average, with 10 remaining intact at the 40 second mark, by which time all the Apex pods had broken. The resistance of 997 is described as intermediate and contrasts with the results for POSH selection 590 given in the lower graph. Here, breakage is delayed beyond that of 997 but there is a further effect in that the proportion of unbroken pods differs from those which are broken but remain intact - these became known as 'hangers' because although the pods were split, there remained sufficient resistance within the pods to prevent then shattering completely. Thus, although these pods were split, they remained intact enough to retain their seeds and were, therefore, technically not shattered. However, in

selecting for good levels of psr, it was considered desirable to choose plants with completely unbroken pods rather than the 'hangers', whilst recognising the potential these latter types might have in helping to overcome the problem of pod shatter in practice.

DISCUSSION

All the POSH 1 F2 families segregated for the abnormal phenotype of reduced petal with a severe fold and poor pollen production. The ratio was consistent across all the 6 families, giving an average ratio of 1:4.2. This ratio is highly significantly different to 1:3 (single recessive gene model) suggesting either that the reduced petal individuals are less fit and some die before being scored or that segregation in the gametes is non-Mendelian or that the trait is under complex control of more than one locus. F2 progenies with normal flowers and pollen were recovered and some of these did produce mature pods showing normal fertility and also pod shatter resistance. This demonstrates that the pod shatter trait is recoverable in segregating material and further that the most serious defect in the synthetic parental material (poor fertility) are not pleiotropically associated. It may be concluded that further backcrossing of this material to Apex will make progress towards improving the agronomic performance whilst retaining shatter resistance.

REFERENCES

Peterson M, Sander L, Child RD, Van Onckelen HA, Ulvskov P and Borkhardt B. (1996) Isolation and characterisation of a pod dehiscence zone-specific polygalacturonase from Brassica napus. Plant Molecular Biology 31: 517-527.

Table 1. Segregation of the abnormal reduced petal phenotype in F2 progenies CPBT data

POSH F1 individual	narrow petal, severe. fold and poor pollen	normal flower		Chi-squared test against 1:3 ratio	
POSH 1-1	9	43		**0.13**	ns
POSH 1-2	41	150		**0.57**	ns
POSH 1-3	62	247		**0.11**	ns
POSH 1-4	39	199		**1.31**	ns
POSH 1-5	13	54		**0.00**	ns
POSH 1-6	17	63		**0.19**	ns
TOTAL	**181**	**756**			
	0.19	0.81			

Table 2.

Summary of MIT scores from the CPBT F2 POSH Populations grown in field 2000

	POSH 1-1	POSH 1-2	POSH 1-3	POSH 1-4	POSH 1-5	POSH 1-6	POSH 2-1	POSH 2-2	POSH 2-3	POSH 2-4	POSH 1 total	POSH 2 total	Overall total	Apex
Tot plants	400	450	630	500	250	250	380	370	280	240	2480	1270	3750	
selected at FT	83	115	92	134	26	29	74	71	69	84	479	298	777	15
% selected	20.8%	25.6%	14.6%	26.8%	10.4%	11.6%	19.5%	19.2%	24.6%	35.0%	19.3%	23.5%	20.7%	
mean MIT score (1-9 scale)	2.35	2.43	2.15	2.19	2.00	2.00	2.42	2.74	3.16	2.15	2.25	2.59	2.38	2.00
range 1-2	60	92	83	109	24	28	62	53	41	73	396	229	625	15
range 3-5	15	18	7	10	2	0	6	9	16	10	52	41	93	0
range 6-9	**2**	**4**	**1**	**3**	**0**	**0**	**6**	**8**	**12**	**1**	**10**	**27**	**37**	**0**
missing	6	1	1	12	0	1	0	1	0	0	21	1	22	0
range 7-9 as %	2.60%	3.51%	1.10%	2.46%	0.00%	0.00%	8.11%	11.43%	17.39%	1.19%	2.18%	9.09%	4.90%	0.00%
range 7-9 as ratio	1:38	1:28	1:90	1:40			1:11	1:8	1:5	1:83	1:45	1:10	1:19	
set on harvested plants	6.50	4.29	8.00	6.00			5.00	5.25	5.75	2.00	5.31	5.07	5.29	8.00

Table 3. Characters scored on F2 POSH populations June/July 2000

Flowering time	1-6 weekly, 1= first to flower
Flower colour	1=yellow 2=primrose 3=cream 4=white scored on the young fully open flowers.
Vigour	1=weak 2=small 3=medium 4=strong 5=very strong assessed in terms of gross bulk of the plant.
Branch Angle	1=very flat(lowest branches along ground) 2=flattened 3=45 degrees 4=30 degrees 5=erect
Branch No.	1=very few branches 2=less branches than normal 3=moderate branching 4=many branches 5= lots of branches becoming bush like. (Branching has been taken to include an average across all branches.)
Stem	Stem thickness on a scale of 1 weak to 5 very robust
Fasciation	1=none 2=very mild, paired pedicels etc 3=obvious 4=bad, several stems affected, narrow strap stems 5=severe, very obvious effect on one or more stems.
Leaf Colour	1=pale and yellowish 2=pale green 3=mid green 4=dark green 5= very dark. (Apex typically 4-5 on this scale).

POSH Line	Flowering Time	Flower Colour	Vigour	Branch Angle	Branch Number	Stem Diameter	Fasciation	Leaf Colour	MIT
1.1	3.7 0.85	1.0	3.3 0.79	3.3 0.82	3.0 0.57	3.4 0.62	1.0	2.9 0.70	2.4 0.76
1.2	3.4 0.86	1.0	3.2 0.61	3.3 0.66	2.8 0.60	3.2 0.50	1.0	2.8 0.63	2.1 0.44
1.3	3.3 0.78	1.0	3.0 0.74	3.3 0.74	3.0 0.51	3.1 0.47	1.0	3.0 0.66	2.0 0.62
1.4	3.5 0.77	1.0	3.2 0.65	3.5 0.75	3.0 0.52	3.1 0.45	1.0	3.0 0.67	2.3 0.58
1.5	4.0 0.90	1.0	3.1 0.81	3.5 0.95	3.0 0.58	3.2 0.54	1.0	3.2 0.80	2.0 0.42
1.6	3.7 0.89	1.0	2.9 0.86	3.4 0.76	3.2 0.68	3.0 0.41	1.0	3.0 0.75	1.9 0.52
2.1	3.4 1.10	2.5	3.0 0.64	2.8 0.78	3.2 0.67	3.0 0.54	1.2	2.5 0.79	2.1 0.57
2.2	3.9 1.10	2.5	3.0 0.70	3.3 0.89	3.1 0.72	3.1 0.57	1.5	2.5 0.69	2.3 0.79
2.3	3.7 0.92	2.2	3.0 0.76	3.3 0.85	3.2 0.64	3.1 0.60	1.7	2.5 0.69	2.1 0.60
2.4	3.6 1.03	2.3	2.8 0.76	3.5 0.81	3.3 0.69	3.2 0.53	2.2	2.5 0.65	1.8 0.68

Table 4. Means (above) and standard deviations (below - in each cell) for the F2 POSH lines grown in the field in 2000.

Traits are as described in Table 3

(Note: no standard deviations are given for Flower Colour or Fasciation because the distributions for these two traits were significantly skewed from Normal.)

POSH Line	F2 Code	Pedicel Length	Pod Length	Pod Depth	Pod Width	Beak Length	Pod Angle	Seeds /Pod	Mean Seed Wt	RIT (40s)	RIT (80s)
1.1	37	25.6 0.60	70.0 2.85	4.5 0.13	3.52 0.11	10.2 0.97	57.8 7.45	24.2	2.31	4	0
1.1	76	32.6 1.08	77.8 4.21	5.52 0.13	3.46 0.04	16.0 1.41	40.6 8.09	22.8	4.75	10	1
1.2	1850	24.6 0.51	71.4 0.93	5.12 0.15	3.32 0.07	12.4 0.75	41.6 5.57	21.0	6.31	13	4
1.2	1853	29.6 1.12	72.8 1.24	5.54 0.09	3.38 0.09	14.6 0.68	38.6 2.64	23.2	5.39	15	7
1.3	590	27.8 1.02	60.8 2.13	5.72 0.13	3.90 0.05	12.8 0.37	51.2 2.58	17.6	7.17	20	15
1.3	597	37.8 2.67	49.2 4.43	5.42 0.17	3.98 0.07	14.4 1.03	37.2 5.20	11.6	6.76	13	4
1.4	997	20.0 0.71	56.0 1.76	4.12 0.04	3.22 0.06	14.8 0.37	49.8 6.21	13.2	5.18	15	7
1.4	1133		51.8 3.29	5.00 0.16	3.40 0.06	14.8 0.76		13.6	4.87	13	7
2.1	1448		47.2 1.74	6.48 0.09	3.84 0.10	12.0 0.32		13.6	5.88	7	0
2.2	333							7.4	7.35	12	1
2.2	351		57.6 2.01	5.08 0.16	2.96 0.07	13.2 0.92		14.4	4.75	16	3
2.2	352		53.6 1.10	5.96 0.10	4.30 0.16	15.8 0.73		9.8	5.47	15	4
2.3	1587	33.4 1.94	47.4 1.50	5.32 0.14	3.42 0.13	17.6 0.68	39.8 5.17	19.0	4.78	5	1
Apex		23.8 0.63	60.9 0.99	4.70 0.08	2.87 0.04	14.6 0.40		27.1	4.41	0	0

Table 5. Summary of pod related traits for lines selected from amongst the F2 population for good field pod shatter resistance

POSH Line	Number Individuals Tested	Average No. Intact Pods after 40 secs	Average No. Intact Pods after 80 secs
1.1	13	1.23	0.00
1.2	6	2.67	0.67
1.3	24	2.96	0.38
1.4	13	2.15	0.23
1.6	2	0.00	0.00
2.1	6	1.83	0.00
2.2	33	2.94	0.30
2.3	13	0.69	0.00
Apex	8	0.13	0.00

Table 6. RIT results for 20 pods taken from each of 110 F2 selections and Apex grown in the field in 2000.

Flowering Time	1.00								
Flower Colour	0.05	1.00							
Plant Vigour	0.04	-0.11	1.00						
Branch Angle	0.21	-0.15	0.03	1.00					
Branch Number	-0.08	0.18	-0.09	-0.01	1.00				
Stem Thickness	0.06	0.02	0.20	0.00	0.19	1.00			
Fasciation	0.05	0.26	-0.12	0.07	0.14	0.10	1.00		
Leaf Colour	0.21	-0.21	0.18	0.13	-0.04	0.04	-0.10	1.00	
RIT	0.08	0.00	0.14	0.04	-0.07	0.10	-0.03	0.06	1.00
	Flowering Time	Flower Colour	Plant Vigour	Branch Angle	Branch Number	Stem Thickness	Fasciation	Leaf Colour	RIT

Table 7. Correlations amongst traits scored on the F2 population grown in the field in 2000. All families combined.

Fig 1: Origin of the synthetic parents

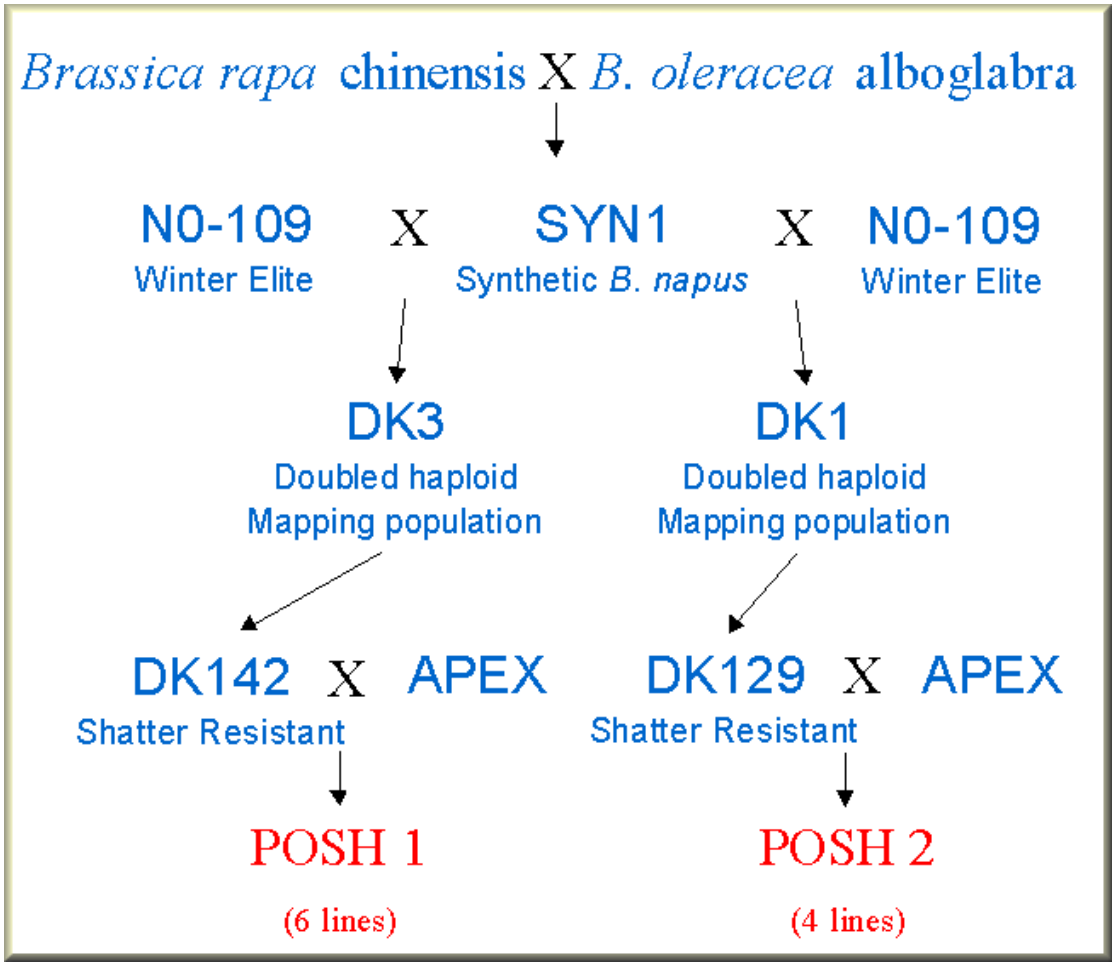
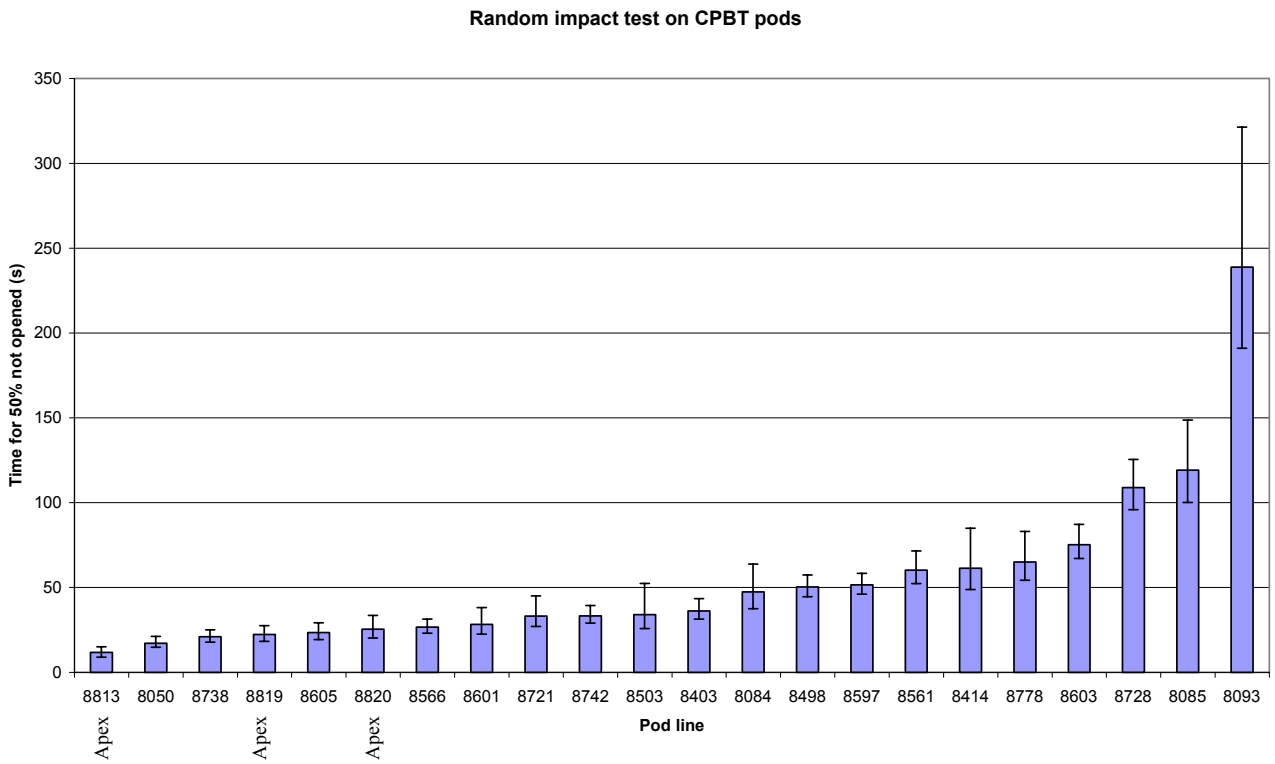


Fig 2 : Distribution of CPBT selected F2 individuals tested using the random impact test (RIT)



Introgression of pod shatter resistance into commercial breeding material: II Identification and analysis of undesireable traits

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INTRODUCTION

The pod shatter resistance sources DK 142 and DK 129 are derived from synthetic reconstructions of the *B.napus* genome. It is commonly found that such synthetic introductions of novel traits also introduce undesireable phenotypes as co-incidental associated genetic material. These unwanted events can fall into two broad categories, A) Genetic factors not linked to the genes controlling the target trait, these usually segregate independently of the target trait and can therefore be eliminated during the course of a recurrent backcrossing programme. B) Chromosomal rearrangements relative to the genome of commercial *B.napus*, these can cause minor or major problems during meiosis leading to unbalanced gametes. The unbalanced gametes may lead to offspring suffering from deletions or duplications and therefore showing abnormal phenotypes and/or reduced fertility. In the present study both parental lines DK 142 and DK 129 although doubled haploid lines produced from microspores were known to be unstable and to generate variation upon selfing. The POSH_1 and POSH_2 F1 hybrid progenies were also observed to be slightly variable. These problems suggest that rearrangements could be present in the material used to produce the doubled haploid mapping populations used in this study. For the purposes of introgression rearrangements may be no obstacle provided the gene(s) controlling the target trait are not located on a region of the genome affected by the rearrangement. C) The successful introgression of the target phenotype may be compromised and certainly will be more difficult if there is a genetic linkage between one or more of the target genes and an undesireable genic factor or rearrangement. The identification and study of the segregation of undesireable phenotypes at an early segregating generation can often prove valuable in showing the presence of difficult linkages.

MATERIALS AND METHODS

A sub-set (132) of the POSH_1 and POSH_2 MC lines (determined by availability of a minimum of 15 seeds in excess of the requirements for the main trail at JIC) were direct drilled into the field at CPBT in the autumn 2000. Seed quality was very variable and establishment of many lines was poor. Many lines continued to show poor vigour compared to conventional material and therefore winter losses were greater than for normal material. In total 28 lines failed entirely (21%).

During the geen pod stage the lodging of the plants was assessed using a 1-9 point scale (where 1 represents complete lodging and 9 fully erect). At maturity lodging stem canker (*Leptosphaeria maculans)* was assessed on a 1-9 scale (where 1 indicates severe disease and 9 no disease symptoms). Shatter resistance was scored using the MIT procedure and scored on a 1-9 scale, this has been rescaled to the 1-5 point scale and is shown in the results table. Pods were scored in early August at least 10 days after the completion of the harvest of all conventional breeding material in the same field. Pods were completely ripe with no signs of greenness. Two samples of racemes were collected from each row, one contained a single main or primary raceme the other several racemes. These were used to provide intact pods for random impact testing (RIT).

RESULTS

CPBT results for the Doubled Haploid Lines

The DH lines segregated for all the traits assessed, the distribution for each of the traits is shown in Figures 1-4. In each case lines were produced which showed more extreme phenotypes than the parent Apex. The MIT scores showed a few lines with significantly improved shatter resistance. The stem canker incidence was severe in its effects and led to early death of the most affected plants. The stem canker assessment gives a strong indication of a bimodal distribution suggesting that a single major gene is segregating in the population. The parent Apex is essentially resistant indicating that an allele conferring susceptibility to stem canker has been introduced from the synthetic parents. The seed set distribution shows that, as found for the F2 populations, many lines do suffer a substantial reduction in fertility. However, there are nearly 40% of lines with seed sets equal to Apex.

Table 1 shows the correlation coefficients between each of the traits. The strongest of these is between stem canker and lodging, in this case this probably is cause and effect with the occurrence of stem canker leading to lodging. Field notes taken during the season support this interpretation. Similarly the positive correlation of 0.3 between stem canker and seed set is probably the effect of severe canker leading to poor pod fill / seed abortion. The critical correlations are between the measure of pod shatter resistance (MIT) and the stem canker and seed set scores. For neither of these is there an indication of any significant relationship.

JIC results for the Doubled Haploid Lines

By the time the data from the doubled haploid lines grown at JIC during 2001 was available for analysis, the Consortium had already agreed to concentrate on the POSH1 population at the expense of POSH2. Thus, statistical analyses were carried out on the data from the POSH1 doubled haploids as a priority and results are presented and discussed here.

Table 2 provides means and standard deviations for the POSH1 doubled halpoid lines grown at JIC during the 2001 season, with a selection of the traits assessed. Results from two control cultivars, Apex and Synergy were included for comparative purposes. The means and standard deviations are based on five replicates in each of two blocks for each of the individual lines within the POSH1.1 to POSH1.6 set. Thus, although POSH1.4 is indicated as being represented by only 5 individuals in Table 2, the experiment did include ten replicates for each of these to provide adequate estimates of the means and variability.

For Flowering Time, the POSH lines tended to flower earlier than the controls but the range within lines was greater - as expected, since each line contained a number of genetically different individuals. Rosette and Mature Vigour, and Leaf Colour all gave slightly lower values than the controls but were, generally, similar. As with the POSH F2 selections (Werner et al. I), the Mean Seed Weights tended to be higher than the cultivar controls but Seed Number per Pod lower. The MIT scores were mostly disappointing, with only three of the five lines giving values higher than the controls, and the two remaining POSH lines were lower; however, some individuals within the lines gave higher MIT scores, as they did for the RIT. Here, it is interesting to note that POSH1.3 gave an equal best RIT score but also gave the highest standard deviation, indicating that the range within the line was greater and it did, indeed, include some individuals with higher levels of psr. The RIT values for the controls were low, with a much reduced range within, as expected.

Correlations amongst these traits were low (Table 3), demonstrating that these traits are largely independent of each other - a situation similar to that found amongst the POSH F2 selections (Werner et al. I). In particular note the low correlations between the seed weight and number traits and the pod shatter traits. The two higher value correlations of r=0.47 for Rosette and Mature Vigour, and r=0.58 for MIT and RIT are to be expected - indeed, it may be surprising that these latter two measures of psr are not associated more strongly with a higher correlation value. These intermediate values for the correlation between RIT and MIT have been consistent throughout and have been, at best, no more than r=0.7. Whilst developing and investigating the various methods for assessing psr, this was cause for concern amongst the Consortium but as our understanding of the mechanism of resistance improved, it was realised that, although providing useful measures of resistance, the two tests are, in fact, assessing different aspects of resistance and, as such, may well be expected to be not so highly correlated. Further information as the SRI and LARS work progressed confirmed this. Thus, whilst both tests have their respective uses - the MIT being very readily applied on a large scale for selecting in the field, and the RIT being a carefully controlled, repeatable and reliable assessment carried out in the laboratory but very labour intensive and time consuming - it should be recognised that they do, in fact, assess somewhat different aspects of resistance.

The correlations presented in Table 3 are for all families within lines combined - results from the lines taken separately are essentially similar with no significant departures from the results in our earlier paper on the F2 population (Werner et al. I).

DISCUSSION

The observation of deleterious traits segregating in this material was not unexpected. It was certainly unfortunate that susceptibility to stem canker is segregating but the results suggest that this under the control of a single major gene and that this is not linked to the target pod shatter resistance. If this is correct then it will prove possible to remove the susceptibility during the introgression process using a combination of selection and further backcrossing. The problem of reduced fertility may indicate an underlying rearrangement within the genome which could be more difficult to deal with. However, there are a good number of lines segregating that have normal fertility and there is no correlation between the fertility and the pod shatter resistance, this indicates that it is unlikely that the gene(s) controlling the pod shatter resistance are involved in any rearrangements. These phenotypic observations leading to a suspicion of the presence of rearrangements may be reviewed when genetic mapping information becomes available.

Fig. 1. Distribution of the DH lines: Manual Impact Test (MIT) assessment of shatter resistance

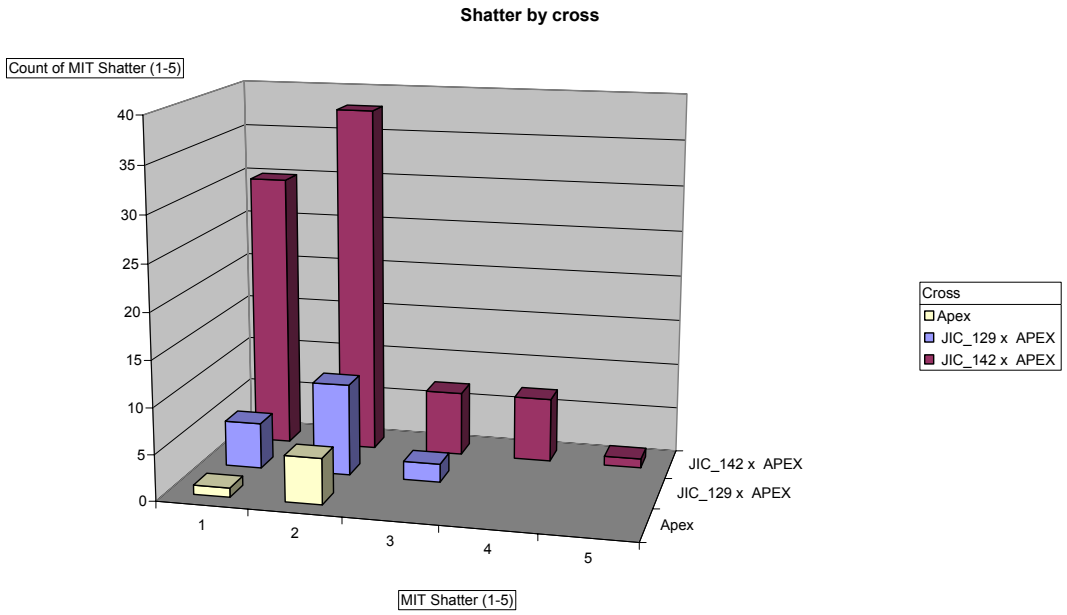


Fig. 2. Distribution of the DH lines: Lodging

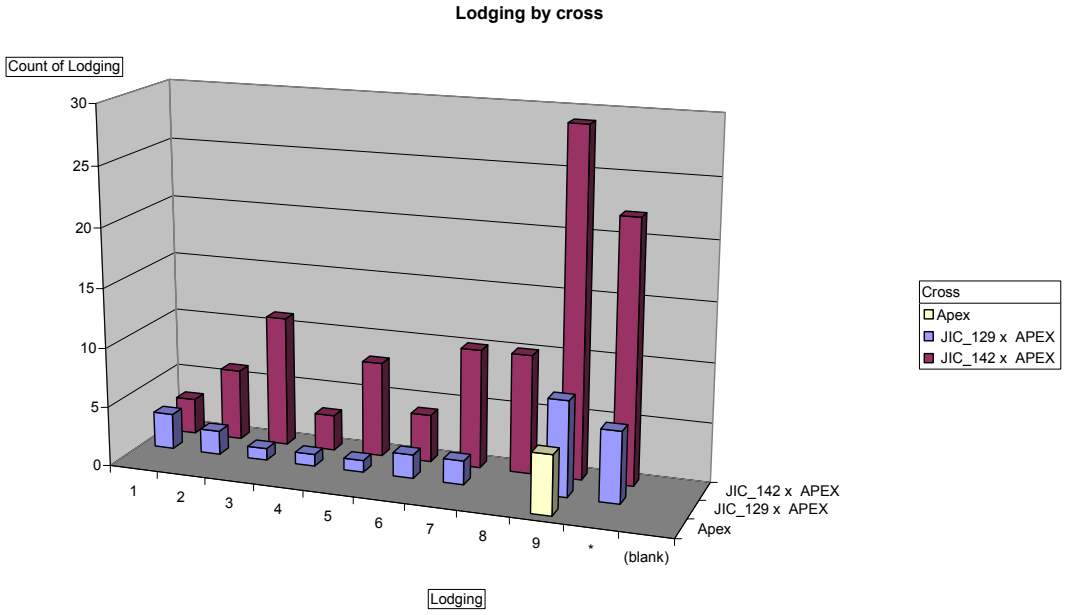


Fig. 3. Distribution of the DH lines: Stem canker infection

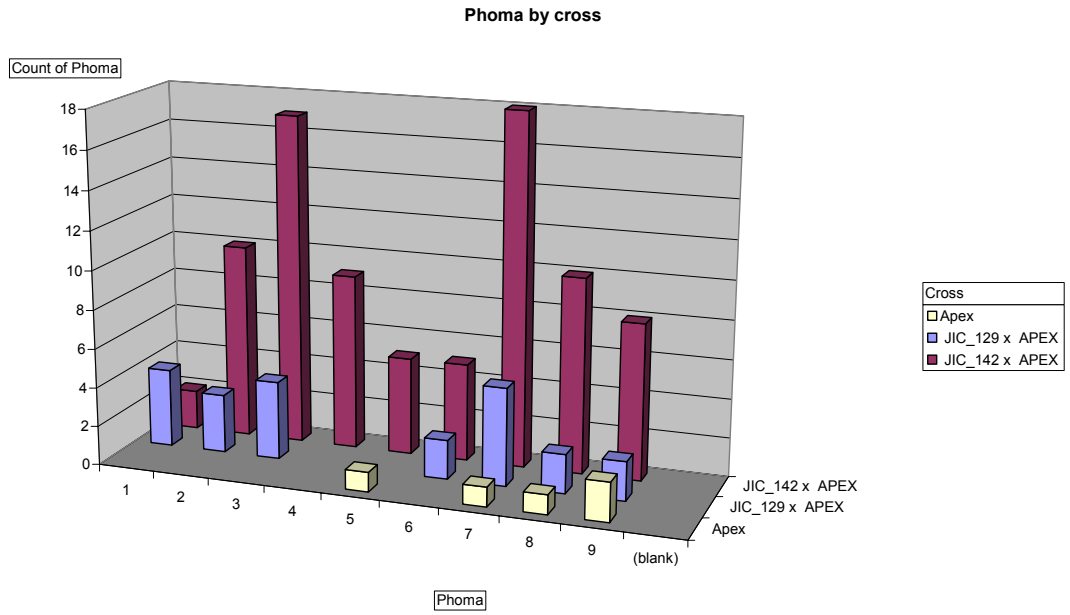
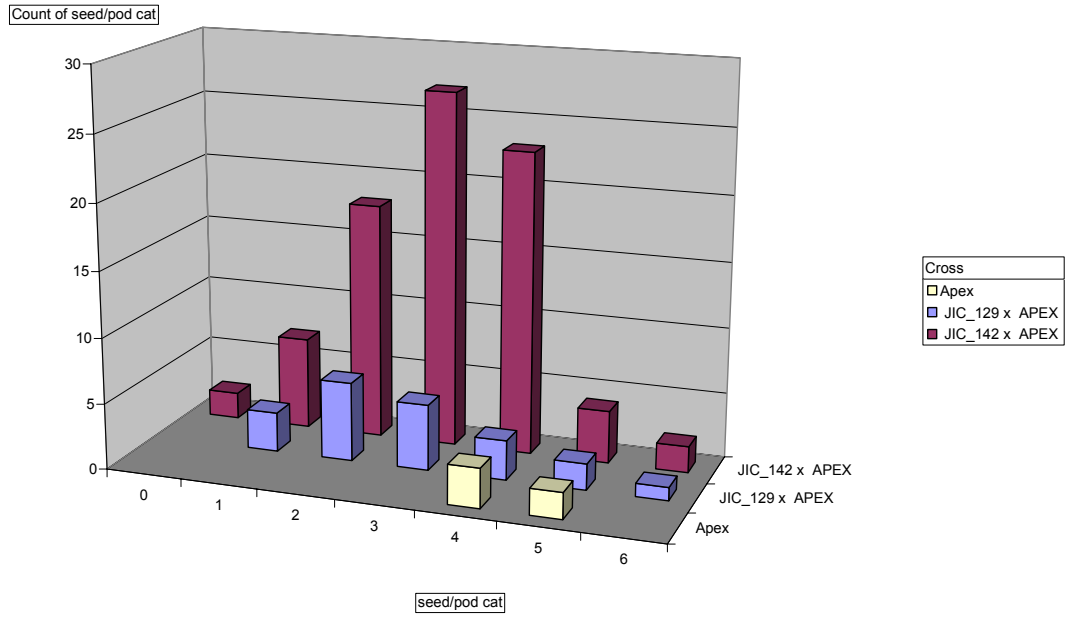


Fig. 4. Distribution of the DH lines: Seed set(seeds per flower by category: 0=0-5, 1=5-10, 2=10-15, 3=15-20, 4=20-25, 5=25-30, 6=30-35)



	Lodging	*Stem canker*	*MIT Shatter*	*Mean seed /pod*
Lodging	1.000			
Stem canker	0.738	1.000		
MIT Shatter	-0.074	-0.102	1.000	
Mean seed /pod	0.183	0.336	-0.154	1.000

Table 1. Correlations between the traits assessed for the DH lines (96 lines) at CPBT in 2001

POSH Line	Number Individuals per Line	RIT	Mean Seed Wt	Seeds per Pod	Flowering Time	Rosette Vigour	Leaf Colour	Mature Vigour	MIT
1.1	24	13.5 7.47	4.67 0.91	15.6 6.05	21.1 4.69	3.72 0.78	2.87 0.75	2.94 0.62	1.80 0.70
1.2	18	18.2 11.7	4.79 0.73	15.4 8.25	21.1 3.73	3.64 0.89	2.52 0.69	2.59 0.80	1.97 0.88
1.3	68	18.2 14.2	5.21 0.96	13.6 6.68	18.8 4.73	3.57 0.88	2.94 0.75	2.49 0.67	1.81 0.81
1.4	5	9.2 3.34	4.94 0.82	12.2 8.10	23.3 2.68	3.90 0.94	3.37 0.76	3.00 0.69	1.39 0.50
1.6	40	8.52 4.67	4.56 0.94	12.5 6.63	19.7 4.41	2.32 0.77	2.76 0.61	2.49 0.68	1.31 0.53
Controls (Apex + Synergy)	2	8.04 2.70	4.26 0.70	25.3 2.65	26.3 1.49	2.75 0.46	3.00 0.76	3.63 0.74	1.50 0.76

Table 2. Means (above) and standard deviations (below) for POSH1 Doubled Haploid lines grown at JIC in 2001, with cvs Apex and Synergy as controls.

Mean Seed Wt	1.00							
Seeds per Pod	-0.22	1.00						
Flowering Time	-0.08	0.10	1.00					
Rosette Vigour	-0.01	0.22	0.07	1.00				
Leaf Colour	0.02	0.02	0.13	0.12	1.00			
Mature Vigour	-0.08	0.36	0.32	0.47	0.13	1.00		
MIT	0.20	0.02	0.14	0.16	0.06	0.25	1.00	
RIT	0.26	-0.04	0.14	0.16	0.13	0.18	0.58	1.00
	Mean Seed Wt	Seeds per Pod	Flowering Time	Rosette Vigour	Leaf Colour	Mature Vigour	MIT	RIT

Table 3. Correlations amongst traits scored on the POSH1 Doubled Haploid lines grown in the field at JIC in 2001. All families combined.

Introgression of pod shatter resistance into commercial breeding material: III Progress towards varieties

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INTRODUCTION

Oilseed rape (*B. napus*) is the most important oilseed crop grown in temperate agricultural regions but it is subject to significant losses of seed as a result of premature dehiscence before and during harvest. This loss is typically 8-12% of the total seed yield (Kadkol *et al.,* 1984) but can increase to over 20% if harvesting is delayed beyond the optimum time (Price *et al*., 1996). It is estimated that this loss of seed can accumulate to reach 10,000 seeds/m^2 on the soil surface (Lutman, 1993) and leads to the contamination of subsequent crops by the resulting volunteer plants. It has been estimated that 23% of winter cereal crops are contaminated with oilseed rape in the U.K. (Whitehead and Wright, 1989). These volunteer oilseed rape plants may simply act competitively as a weed but may also cause genetic contamination, as a result of cross pollination, if the following crop is a different cultivar of oilseed rape. This may be particularly important where, for instance, a low erucic acid cultivar follows a high erucic crop. This problem is exacerbated as seed of oilseed rape may remain dormant in the soil for many years. Additionally, lack of developmental synchrony in modern cultivars means that first-formed pods open and shed mature seeds whilst later-formed pods still contain immature seed. Improvement in the uniformity of maturity at harvest is attempted by swathing or spraying with desiccants which, however, also results in the chlorophyll of immature seeds contaminating the extracted oil and hence lowering its quality. Increased shatter resistance will promote natural maturing of uniformly ripe seeds with improved oil extraction characteristics. Production costs, efficiency of seed recovery and quality of oil would all be improved by increased shatter resistance.

Shattering takes place following dehydration of the pod wall and separation of the cells in a dehiscence zone which is situated in sutures between the lignified pod wall edge and a replum containing vascular tissue (*e.g.* Picart and Morgan, 1984). The dehiscence zone cells separate along the line of the middle lamella, following degradation of the pectin by polygalacturonase (Petersen et al., 1996) and subsequent breakdown of the dehiscence zone cell walls. The pods open as a result of the application of external forces supplied by contact with other pods, racemes or harvesting machinery which severs the vascular connections which pass across the dehiscence zone from pod wall to the replum.

There is little variation for resistance to shattering within existing breeding programmes of *B. napus* but resistant lines have been found within the diploid parents of *B. napus* (*B. oleracea* and *B. rapa*) as well as within other members of the *Brassicae*, notably *B. juncea, B. carinata* and *B. nigra* (*e.g.* Kirk and Hurlstone, 1983). Papers covering the physiology and morphology of the pod shatter resistant material forming the basis of the current study (Summers *et. al.,* 2003 and Child *et.al.*, submitted) have shown that the parental line DK 142 primarily shows shatter resistant properties owing to an increased main vascular bundle entering the pod valve at the replum. This extra tissue is simply harder to crack and therefore the explosive shattering of the mature valve cannot be easily initiated. The genetic architecture of this trait is not however currently understood and may therefore be under simple genetic control involving one or two loci but could be much more complex involving many genes of small effect.

Whilst this introduced modification forms the basis of the main gain in shatter resistance it is known that shatter resistance is influenced by other factors (Thurling, 1991). These include morphological characteristics of both the whole plant and raceme as well as those of single pods and how individual characters relate with each other. Within the crop canopy, before and during harvest, much pod shattering occurs because of the natural movement of the canopy which results in pods knocking against each other or against the stems and branches. This problem of mechanical damage is likely to be much affected by other plant attributes such as pod angles, pod length and width. Together with other aspects of plant architecture such as height and stem stiffness, these attributes may affect the laxness of the plant and hence the degree and type of movement made by the canopy and of branches within it (Loof and Jonsson, 1970; Thompson and Hughes, 1986).

In this paper we will describe the progress made with the introduction of shatter resistance from the synthetic sources DK 142 and DK 128 into a commercial background. As described in the earlier paper (Werner *et.al.* II) these parental lines introduced several deleterious traits that require removal whilst retaining the PSR.

MATERIALS AND METHODS

From amongst populations of synthetic *Brassica napus* produced at the John Innes Centre, two synthetic doubled haploid lines were identified as having particularly high levels of pod shatter resistance (PSR) when grown as part of a larger experiment designed to assess variation on this novel material. These lines, coded DK142 and DK129, were subsequently included in experiments to investigate the genetic control of the PSR trait (described in the paper by Morgan et al, 2000), and contributed to the SAPPIO Link programme for use in this project. Their derivation and use in this project is illustrated in Fig. 1. The two PSR synthetic doubled haploid lines were used as female parents in crosses with cv Apex to produce F1 progenies POSH1 and POSH2.

A number of different strategies were employed to enhance the development of material showing PSR. These were carried out in Cambridge at CPB Twyford Ltd (CPBT), in Gent at Bayer Crop Science (Gent) and at the John Innes Centre in Norwich (JIC). Fig 2. shows the structure of the introgression work.

At CPBT F3 lines derived from F2 individuals selected in 2000 were grown in the field. The most promising of these (based on the various shatter scores from the F2 pods) were backcrossed in the field or the glasshouse to Apex. Up to 3 individuals were backcrossed for each family. The F3 individuals were tested at maturity for their PSR using the field based manual impact test (MIT), the backcross seeds from individuals with MIT scores >5 were sown in the glasshouse and self pollinated to produce the $[F3,B1]^2$ generation (a total of 3 combinations tracing to 2 F2 individuals). These were grown on in the glasshouse using single seed descent (SSD) to produce a total of 203 lines at $[F3,B1]^3$.

At the JIC 4 promising F2 individuals were selected and between 50 and 170 F3 individuals per family were backcrossed to Apex. These [F3,B1] progenies were grown in a glasshouse and self pollinated to produce segregating populations of $[F3,B1]^2$ seed. 25 families derived from each F2 were selected and a total of 2,629 transplants of the $[F3,B1]^2$ generation plus controls were planted in 4 blocks, of linearly arranged families, with plants spaced in sub-blocks of 10. Owing to the lateness of transplantation (June 2002), pods reached the required state of maturity for shatter evaluation (MIT) late in the season but did enable good selection for PSR to be made.

The approach in Gent was to continue pedigree selection from the original F2 populations to the F5 generation. The BCS strategy has been to fix the podshatter resistance trait derived from this material through a selfing & trait-scoring selection scheme. To this end 3 field trials have been conducted in three consecutive years. These fields were organized on one location in Belgium in single row configurations, with 2 replicates. Depending on the year, the populations were either sown out in the greenhouse & transplanted in the field, or were directly sown in the field. Self-pollination of the plants was promoted through bagging of the racemes during flowering.

Podshatter resistance was quantified through RIT & MIT analysis. Both methods were performed according to the standard conditions established by other partners in the consortium. D. Bruce (SRI, Silsoe) calculated LD50 values. A lot of effort was spent to standardise the conditions for both podshatter assays.

RESULTS

Observations of the F1 progenies POSH_1 and POSH_2 showed that the PSR is a recessive character. This property together with the expectation that PSR would be under fairly complex genetic control dictated that for introgression purposes it would be necessary to self pollinate and select for PSR among a segregating population. This proved possible in the F2 generation at all three sites CPBT, Gent and JIC (Werner *et.al.* I), but when the F3 seed was regrown the following year it became apparent that many of the selected F2 plants showed only poor PSR or the PSR segregated in the progenies.

CPBT

Following retrospective selection of F3 progenies only 2 F2 individuals were progressed to the backcross progenies stage. These F3 families had improved PSR showing an LD50 for the RIT of 21s and 45s compared to the Apex control of 9s. The seed sets of 19.3 and 22.4 seed/pod for the introgression lines (Apex = 24.6) may not be fully normal but are better than many of the random F2 individuals. The stem canker ratings of 8 and 4 (Apex = 7.5) reflect a real difference between the lineages and clearly the second of the pair can be expected to segregate in the backcross progenies.

JIC

The extreme weather conditions in October 2002 resulted in the spontaneous shattering of many of the susceptible plants, therefore only the resistant end of the range was sampled. Where possible, 40 pods were taken for RIT and in all, pods from 15 of the best plants were sampled.

Gent testing

From the 26 selected F3 lines, the F4 population was grown in a single row configuration on one location in Belgium. Most of the selected lines did no longer display phenotypic abnormalities, as originally observed in the F2 population. Podshatter resistance was quantified both by RIT (Fig. 3) and MIT. The LD50 values of the different lines ranged from 9-38 Sec (Apex control 6-9 sec). The enhanced podshatter resistance of the selected lines has hence been confirmed over several generations. The most interesting selected F4 line is derived from the original POSH2 background, which unfortunately has not been part of the mapping efforts (POSH1).

DISCUSSION

The selection scheme used at Gent to retrieve the better scource for podshatter resistance from the original POSH 1 & POSH2 plant material was based on 3 cycles of podshatter assessment on selfed material. The results from the field trial 2002 clearly demonstrate that the selection scheme works: all the selected F4 lines show enhanced podshatter resistance with different degrees (up to 5-fold) over the control lines. In this selection scheme we have been able to reduce, if not eliminate, most of the undesired agronomic penalties originally observed in the F2 population. This has also been the case with the backcrossing programmes employed at CPBT and JIC.

Progress towards introgression of this trait into commercial breeding material has been slower than hoped owing to the biology of the character. It has not proved possible to identify any associated phenotypes that can be assessed at flowering time or earlier to act that correlate with PSR. It has not, therefore, been possible to avoid treating PSR as a maturity trait and delaying selection until harvest time. The added complication that some degree of segregation for PSR can occur even from a selected individual means that it is necessary to select and backcross with several individuals of the next generation. Each cycle of breeding requires cross, selfing, segregation and selection under field conditions. This results in a minimum breeding cycle time of 2 years per backcross. The value of genetic markers is particularly high for traits of this type since well defined markers that are co-dominant could in oilseed rape lead to a backcross cycle time of 6 months.

Figure 1. Origins of the synthetic parents.

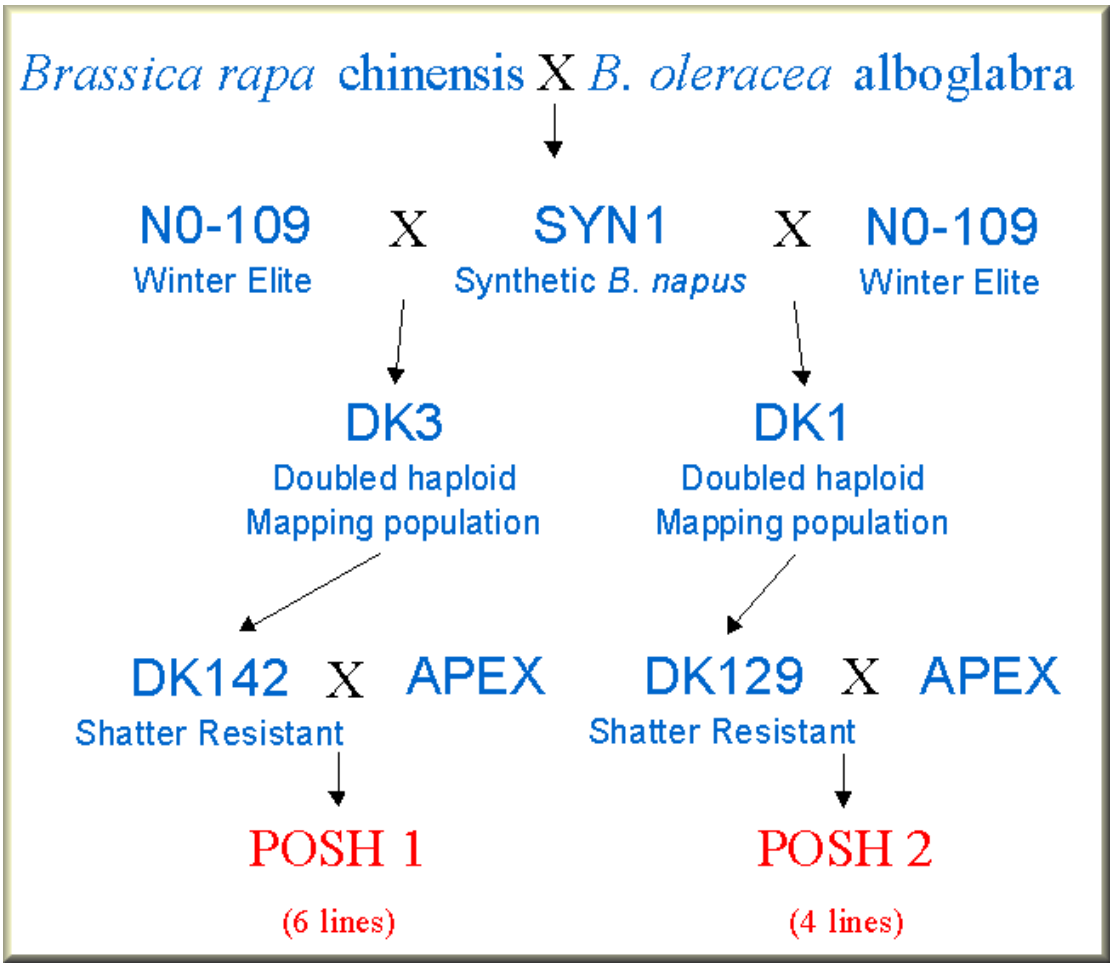


Figure 2. Flow chart of progress

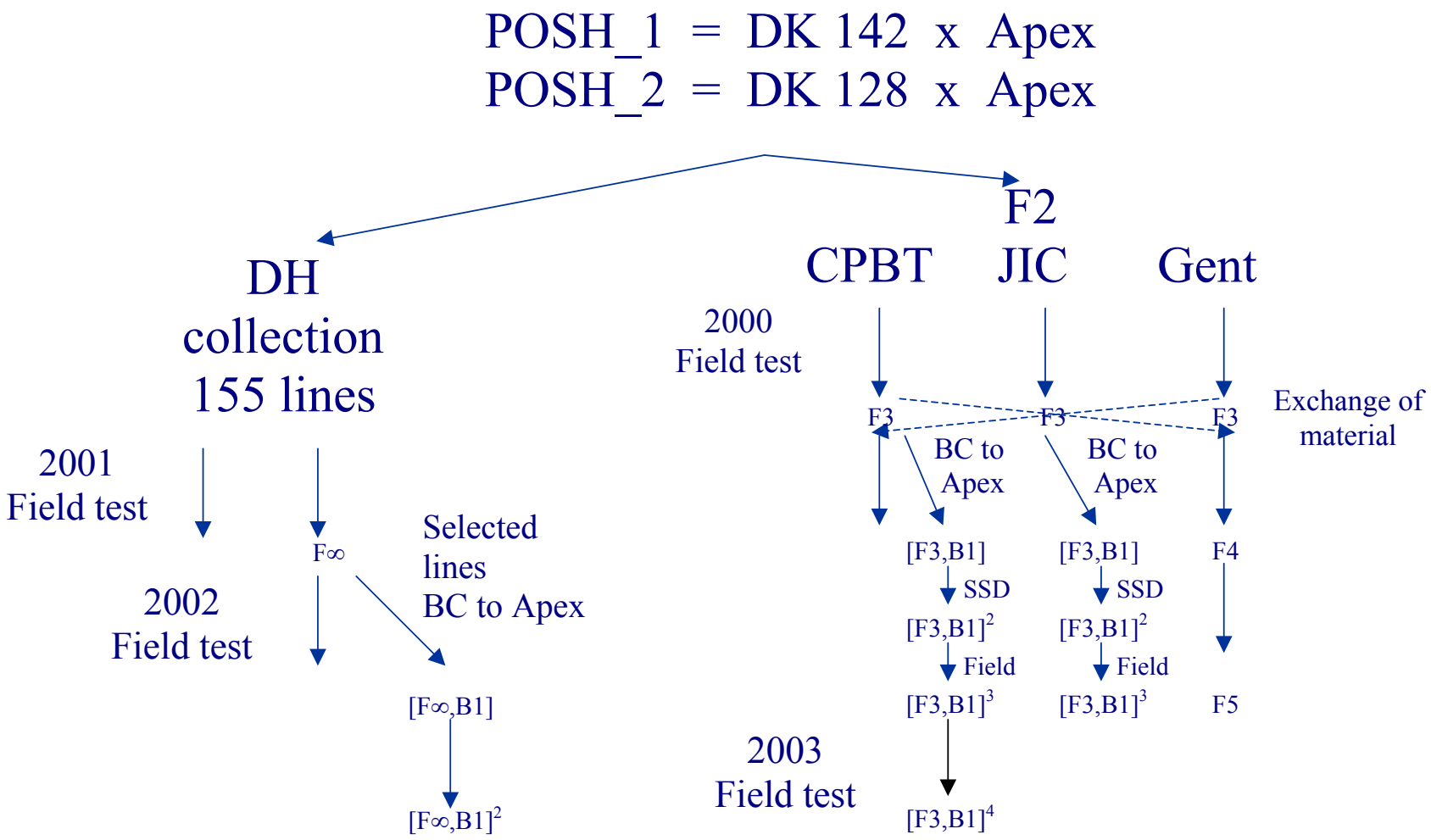
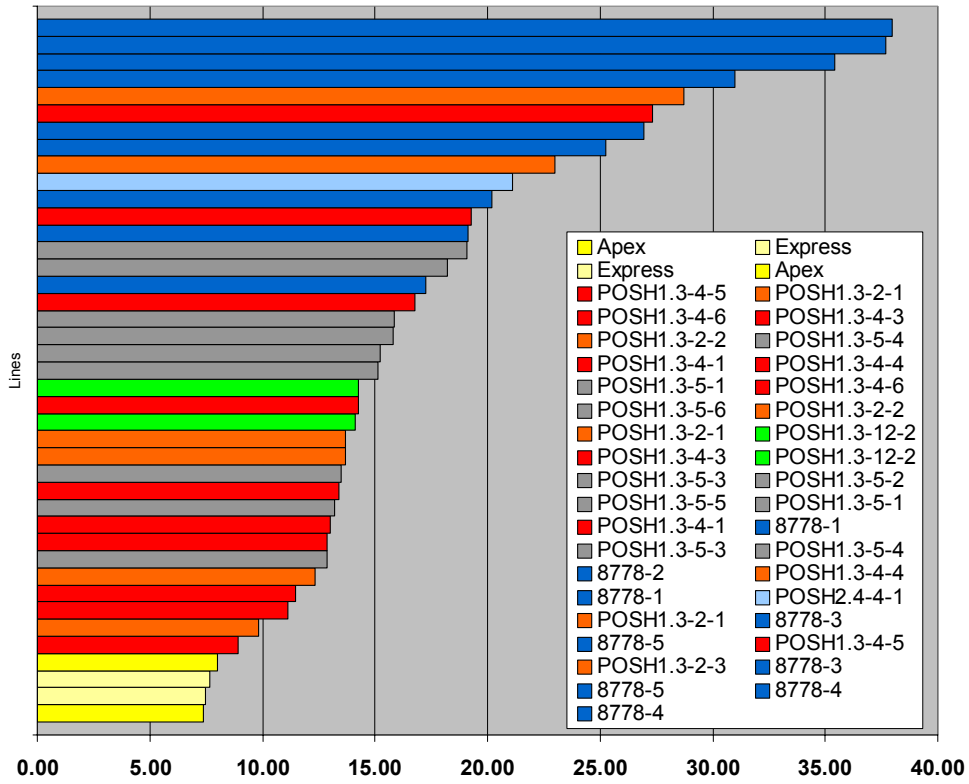


Figure 3. Ranking of all selected F4 lines according to LD50 values as measured on material grown in the field in Gent 2002. Control lines are the cultivars Apex and Express.



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BREEDING OILSEED RAPE FOR POD SHATTERING RESISTANCE

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SUMMARY

The genetic control of pod dehiscence was studied through the production, field trial and subsequent analysis of a full diallel involving seven parents selected for high and low resistance to pod shattering. Additive gene effects were most significant among the measures of pod shattering resistance with only minor contributions from non-additive gene effects. Genetic variation in measures of the stiffness of the pod wall were, however, determined by dominant gene effects. Genes for increased pod shattering resistance acted recessively. All characters showed high levels of heritability. Correlations among pod shatter resistance characters and other pod, raceme and plant characters were low suggesting that resistance is likely to be independent of other important agronomic traits.

INTRODUCTION

Oilseed rape (*Brassica napus* L.) is grown extensively in temperate zones throughout much of northern Europe, northern America and Asia. If it is compared to wheat, however, it is apparent that, in breeding terms, it is relatively undeveloped and in many aspects resembles a weed more than a cultivated crop (Thompson & Hughes 1986). Pods which split easily to facilitate seed dispersal is one of these 'weedy' characters which is extremely detrimental to its use as a crop because considerable amounts of seed may be lost through seed shedding before and during harvest. Estimates of over 20% of the seed yield have been made for this loss (Price et al. 1996) though a typical figure is usually in the region of 10% (Kadkol et al. 1984). The value of the crop within the UK alone amounted to about £420 million p.a. for 1996; thus the loss of 10% amounts to £42 million p.a (MAFF 1997). Potentially, therefore, increases in harvestable seed yield arising from reduced pod shattering will result in significant economic benefits. Additional benefits include the simplification of crop agronomy through the avoidance of swathing (cutting of the stand to promote premature drying), eliminating the use of desiccants, improving uniformity of the harvested seed and the

reduction in seed contamination of the soil. This latter benefit is likely to be of increasing importance as more genetically modified designer crops are grown and strict control measures are needed to avoid cross contamination.

Little variation in resistance to pod shattering has been observed among existing genetic resources or cultivars of oilseed rape so the search for variation has been directed to a broader genetic base through the development of synthetic oilseed rape from wild genotypes of *B. oleracea* and *B. rapa*. This work has been described by Morgan et al. (1998) who also developed several methods of assessing the shatter resistance of individual pods and have also studied several plant, raceme and pod characters that might influence how this resistance is expressed within the crop canopy. Using these data Morgan et al. (1998) identified lines of synthetic rape which had significantly increased resistance to pod shattering. These synthetic lines, however, contained many agronomically deleterious traits including poor seed set and disease susceptibility that made them unsuitable as cultivars. Before attempting to transfer the characters which confer pod shatter resistance into new lines with suitable agronomic characters it is important to understand how these characters are genetically controlled. In this paper we describe the basis of this control through the analysis of the diallel crosses obtained among five lines of synthetic rape and two cultivars. These seven parental lines were selected because they represented a range of the expression of those characters believed to confer pod shattering resistance and also for a variety of other morphological traits.

MATERIAL AND METHODS

Plant material

The five synthetic lines and two cultivars included in the diallel are described in Table 1. They were chosen to represent a range of pod shattering resistance from no resistance (q28) to high resistance (dk142). These parents also differed in many other plant characters including plant height and branching, date of flowering, pod size, shape and angle.

Selfed seed collected from material grown in the field during 1997 was used to produce the parents for the diallel seed production. Seed was germinated in John Innes no. 1 compost and seedlings pricked out into John Innes no. 2 before vernalising for 8 weeks at 6 ^{o}C. The plants were subsequently grown in a glasshouse and bud pollination used to produce a full set of diallel crosses. All crosses were successful though some difficulty in producing selfed seed in lines dk129 and dk142 was noted. The F_1 seed, including all crosses and parents, was then sown and vernalised as above and transplanted into the field in March 1998. Plants were grown at a spacing of 0.5m within rows which were 1m apart in two fully randomised blocks with five replicate plants per block giving a total of 490 plants. Standard agronomic treatments were applied to the trial to prevent pests and diseases at all growth stages including maturation of the pods. The trial was weeded by hand and irrigated when necessary. All plants were staked.

Plant measurements

Pod shattering resistance in a crop is likely to result from the combination of several plant characters including plant and raceme structures affecting canopy architecture, and pod characters affecting the strength of these pods. A range of characters was measured to assess these aspects of plant structure. Date of flowering, taken as the number of days after the first plant to flower (*i.e.* 22nd April 1998), was recorded

during development while just before maturity plant height, basal stem thickness and number of primary branches were recorded for all plants. At maturity a subjective visual and tactile assessment was made of pod shattering resistance of the field grown pods to provide a field score of 0 (very shatter susceptible pods) to 5 (extremely shatter resistant pods). After field assessment the terminal raceme was harvested from each plant and stored in the laboratory. Pod density was determined on each raceme after which 5 typical pods from the middle of the raceme were cut off and pod length and depth, beak length and pod and pedicel angles were measured using a graphics tablet and these data used to estimate raceme width. Mean, minimum and maximum pod wall thicknesses were estimated on one valve (taken at random) per pod using a 'Hall effect' measuring system. In this the pod valve was placed between a fixed and a movable pin which recorded the wall thickness as the pod was drawn between them. The output from the device was recorded on a virtual oscilloscope for analysis. Seed number per pod and mean seed dry weight were then determined on the combined sample of five pods.

Samples of five single mature pods were also harvested and equilibrated to constant humidity before measurement in tensile separation tests (Morgan et al. 1998). In these tests the abaxial surface of single pods were glued to a wooden base and the adaxial surface connected to a universal test machine (Davies & Bruce 1997). A steadily increasing force was applied to these pods and a graph was plotted of force against time from the initiation of the force through dehiscence and then until relaxation of the pod. These data were used to determine the peak load required to initiate dehiscence, the energy needed to complete the fracture of the dehiscence zone and the amount of energy recovered from the pod following bending during the tensile separation tests. Results obtained through the tensile separation tests measure pod attributes affecting dehiscence: peak load measures the force required to make the initial crack at the pedicel end of the pod between the uppermost valve and the replum (as pods naturally dehisce in the field) and is a measure of the strength of the main vascular strand entering the pod and of cell adherence within the dehiscence zone. Fracture energy measures the total energy needed to initiate this crack and to propagate it along the valve-replum interface and is thus a measure of the toughness of the dehiscence zone (both cell adherence and width of the zone are accounted for in its calculation) and any vascular tissue running through this zone. Recovered energy characterises the restoration of the deformed pod valve to its initial shape after tension is released and thus will be affected by the material of the pod wall (conferring stiffness), pod wall thickness and the curvature of the pod. This last is defined by the cross sectional shape of the valve and greater stiffness is likely to be found in deeper cupped u-shaped valves.

Also at maturity one sample of 20 individual pods was harvested from the five replicate plants within each block. These were equilibrated to constant humidity as described above, and used to assess the pod shattering resistance characteristics of the sample by being subjected to shaking in a drum with ball bearings. In this laboratory based, random impact test procedure the number of pods left intact after 20s of standardised shaking in a drum with ball bearings was counted. The test measures the effect of an accumulation of impacts occurring randomly on the pod in contrast to the tensile separation tests which measures the force applied to a specific point, the pedicel end. The random impact tests will therefore differ from the tensile separation tests in being affected by other pod attributes such as pod length and width and any specific weaknesses such as between the beak and the pod valves. Field shatter score is a subjective

assessment of overall pod strength under field conditions and is likely to be more affected by environmental conditions.

Statistical analysis

Preliminary analyses were carried out using individual families (df = 48) and blocks (df = 1) as main effects tested against the amongst replicate plant error (df = 392). Subsequent analyses were carried out to estimate male and female effects (df = 6) and male × female interaction (df = 35) for which the error term included all other interactions (*i.e.* blocks × males, blocks × females and blocks × males × females (df = 392)). Expected mean squares were used to calculate the components of variation from these analyses from which the proportions of the variation attributable to genetic components were estimated. It was noted that values of Φ^2_g ($\Phi^2_{male} + \Phi^2_{female} + \Phi^2_{male.female}$) were highly correlated with Φ^2_{family} obtained from the analysis of family × block (b = +0.916; r = + 0.931) but were, on average 31% higher. These apparently higher values of Φ^2_g resulted from the removal of block effects and interactions from the error term in the second analysis. The values of Φ^2_g derived from the male × female analyses were subdivided into $\Phi^2_{male} + \Phi^2_{female}$ and $\Phi^2_{male.female}$ which were equated with general combining ability (GCA) and specific combining abilities (SCA) respectively.

Five of the traits used to characterise pod shattering resistance were considered for more detailed studies. These included: field shatter score - the basic trait on which the selection of parents in the formation of the diallel was based - and four of the laboratory measures of characters used to assess the trait: the number of pods intact after 20 s from the random impact tests (ip20), the energy needed to cause fracture of the dehiscence zone (fracture energy) and the maximum force needed to initiate dehiscence (peak load) in the tensile separation tests, and the energy recovered from the pods after dehiscence had been achieved. Using the means of the parental arrays in the diallel table, correlation coefficients were calculated among these characters (Fig. 1). Because peak load and fracture energy were very highly correlated and also behaved similarly in further analysis of genetic behaviour, only fracture energy was selected for the subsequent detailed description.

Analysis of variance of the diallel table (Hayman 1954) gave estimates of additive and non-additive gene effects. For these analyses the means of the five replicated plants within each block were used to provide two replicate blocks for this diallel analysis; however, missing experimental plants gave rise to some missing cells within the blocks. To provide a complete data set for the analysis, the data from reciprocal crosses were combined to provide two complete blocks for half diallel analysis. Combining reciprocal data is only valid if there are no maternal, reciprocal effects. These effects were tested for by analysing flowering date, plant height and number of primary branches, characters for which a single matrix with no missing cells was available. The full diallel analysis of these characters showed no reciprocal effects (c) and on this basis it was decided to proceed with the analysis of the half diallel for the selected characters. The error mean square from the family × block analysis of variance, estimating plant to plant variation, was used to provide the tests of significance within the subsequent diallel analyses. Additivity in breeding terms may be equated to narrow sense heritability (also called general combining ability) while broad sense heritability (also called specific combing ability) will also include dominant gene effects. Plots of W_r against V_r were used to test the validity of the Hayman analysis while plots of each hybrid on the mean value for their

parents gave information of general and specific combining ability (additivity and dominance) with respect to individual hybrid families. All analyses of variance, regressions and the Hayman analyses were carried out using the statistical package GENSTAT (Genstat 5, 1987).

RESULTS

Parents

Significant variation among the parents was observed for all the characters measured (Table 2). In general there was about a twofold variation between the most extreme parents for any given character except those relating directly to the measurement of pod shattering resistance where the difference was between five and tenfold. The cultivars, Apex and Tapidor, were characterised by long, horizontal pods resulting in wide racemes in contrast to the pod shatter resistant lines dk129 and dk142 which had shorter, more upright pods. Pod length was associated with seed number per pod though dk150 and especially dk142 had many fewer seeds than expected. There are several possible explanations, not mutually exclusive, for these differences in seed set which include: possible chromosomal rearrangements or deletions following the initial synthesis of the two diploid parent genomes, changes in floral development resulting in late anther dehiscence and poor pollen production, the expression of self incompatability genes (SI) within the genome arising from the synthetic *B. rapa chinensis* × *B. oleracea alboglabra* (Parkin 1995) and the failure of the enzyme polygalacturonase to cause anther dehiscence (Petersen et al. 1996). This enzyme is also associated with pod dehiscence and consequently a pleiotropic linkage between the two similar physiological processes may have resulted in combining resistance to pod shattering with increased sterility and poor seed set. There was no relationship of seed set with mean seed weight. Line dk129 differed significantly from the other lines, including dk142, in having deeper pods with thicker walls, features reflected in the energy recovered from the elasticity of the pod wall measured in the tensile separation tests. The most compliant pods were those of dk129 which retained over four times the recovered energy of the stiffest pods dk150, q28 and Tapidor, with Apex and z79 intermediate for this character.

Parental lines performed as expected for field shatter resistance score such that dk142 was noticeably the most shatter resistant closely followed by dk129. At the other end of the scale q28 had considerably weaker pods. The other parents were intermediate for this character (Table 2). Random impact tests confirmed parent dk142 as the most resistant line though dk129 was similar to the cultivars which appeared relatively shatter resistant compared to the field estimates. As with the field scores q28 was the least resistant followed by z79. Both peak load and fracture energy measured in the tensile separation tests showed that the parents behaved in a similar way to that for field shatter score with large differences apparent between the pod shattering resistant dk142 and the sensitive line q28.

Date of flowering was earliest in q28 and latest in the cultivars and z79 while the dk lines (which included the shattering resistant types) were intermediate. Lines q28 and z79 were the smallest plants as assessed by plant height, stem thickness and number of branches. There were no consistent trends among the other lines for these characters so that dk129 was tallest, dk142 had the thickest stems and Apex had most primary branches.

Field score for shattering resistance

The analysis of variance revealed (Table 3) significant family and block effects though when Φ^2_{block} was estimated as a percentage, its contribution to the total variation was only 5%. The block effect was likely to be the result of changes in temperature and humidity between the occasions when the blocks were assessed. This block effect was thought to have arisen from changes in the weather which occurred between the two occasions when the blocks were measured and which took place over successive days; high humidity may have increased the dampness of the pods resulting in an apparent increased shatter resistance. This illustrates the problems of reproducibility that may arise when using field score as the sole method of assessing pod shatter resistance. There was, however, no family × block interaction as a consequence of this response to the environment. Variation among both male and female arrays was highly significant (Table 4). Additive gene effects ($\Phi^2_{male} + \Phi^2_{female}$) were three times greater than the non-additive component ($\Phi^2_{male \cdot female}$) at 58% and 19% respectively. Results from the Hayman analysis (Table 5) showed both strong additive and non-additive gene effects with a dominance (b) : additivity ratio (a) ($/(H_1/D)$) of 0.58. The dominance (b) contribution to the genetic variation showed significant directional (b_1) and ambi-directional (b_2) components. The reasons for the presence of both these components can be seen in the graph of the relationship between W_r and V_r (Fig. 2a). The regression line did not differ significantly from 1 and lies midway between the 1:1 line and the parabola suggesting the presence both additive and non-additive components. Line dk142 had the highest values of W_r and V_r and was the main determinant of b_1 while the close proximity of the other arrays accounts for the significance of b_2.

The relationship between genetic and phenotypic aspects of the variation are explored in Fig. 2a in which the standardised total mean squares of the array variance and covariance are plotted against the standardised pod shatter scores (data was standardised as $(0 - x) / \Phi^2$. The two shatter resistant lines appear to behave differently in that the high pod shatter resistance in dk142 results from the presence of recessive genes whereas the slightly lower resistance of dk129 appears to depend on moderate dominant gene effects. The other crosses, especially q28, appear to have dominant genes for shattering sensitivity. Fig. 4a shows the relative position of the individual hybrids (cells in the Hayman analysis matrix) as a function of the mean of both parents. The slope of the line is equivalent to additivity (a) in the Hayman analysis or $\Phi^2_{male} + \Phi^2_{female}$ in the male × female analysis of variance. The slope of the fitted regression is less than 1 indicating the presence of non-additive effects (SCA or dominance). The slope is determined chiefly through the hybrids derived from dk129 and dk142 being below the line which also indicates the recessiveness of the character thus affirming the results from the Hayman analysis.

Random impact data --- number of pods intact after 20 s

There were significant differences among families (Table 3) but the absence of replicate data within the blocks did not allow block effects to be estimated. Again, male and female effects were highly significant though there were no male × female interactions. Additive gene effects ($\Phi^2_{male} + \Phi^2_{female}$) were very high (80%) in contrast to non-additive gene effects ($\Phi^2_{male.female}$) which were very low (6%). As with field shatter score, the genetic component (Φ^2_g) was higher than that derived from the analysis of the families. In the Hayman analysis additive gene action was highly significant but non-additive effects were low and ambidirectional (b_2). The dominance ratio of 0.61 was similar to that of field shatter score. This

lack of dominance was also seen in the proximity of the regression line close to the parabola and with a slope which did not differ significantly from 1. It is also seen in the absence of a clear pattern in the distribution of the points in Fig. 3b. A slope of unity in Fig. 4b again shows the additive nature of the genetic control and the absence of points departing from the line also confirms the lack of dominant gene effects.

Tensile separation tests --- Fracture energy

Here, there was a significant family × block interaction, and to a lesser extent block effects. However, as before, these effects were small compared to the family effect and error; thus Φ^2_{family} accounted for 53% of the variation while $\Phi^2_{family.block}$ was only 6%. Variation amongst male and female arrays was again very large with no male × female interaction. Total variation due to genetic factors (Φ^2_g) was very high (69%) with a contribution of 10% from dominant gene effects ($\Phi^2_{male.female}$) to Φ^2_g. In the Hayman analysis dominance was significant and was partitioned jointly between directional (b_1) and ambidirectional (b_2)effects. The dominance ratio was, however, low (0.44) as could be seen from the closeness of the fitted regression line to the parabola in Fig. 1c. As with the field score, the small amount of dominance was for shattering susceptibility in all the lines except those derived from dk142 and dk129 (Fig. 3c). When the hybrid values were plotted against the mean of their parents (Fig. 4c) the fitted regression showed a significant but small decrease in slope confirming the presence of low dominance for the shatter susceptible character. Interestingly, the points in the regression can be split into three groups; those showing low resistance including lines q28 and z79; those showing higher resistance including hybrids derived from dk142 and dk129 and the single hybrid between dk142 and dk129 which had the highest resistance of all.

Recovered energy

There were highly significant differences among the families (Table 3) but these were complicated by the presence of significant family × block interactions which accounted for over 26% of the total variation compared to only 35% accounted for by family effects. The reason for this was not clear. As with the other three characters described above there were highly significant male and female effects but for this character the male × female interaction was largest giving estimates of 28% for dominance effects ($\Phi^2_{male.female}$) compared to 44% for additive effects ($\Phi^2_{male} + \Phi^2_{female}$). These results were reflected in the significant values of both a (additivity) and b_1 and b_2 (dominance) in the Hayman analysis and the high dominance ratio of 0.77. The closeness of the regression to the 1:1 line in Fig. 2d also indicates the presence of dominance gene effects. Plotting the genotypic against the phenotypic expression of this character shown in Fig. 3d shows that the stiffness of the pod wall in dk129 is essentially a recessive character, though Apex, intermediate in this respect, shows dominance. This provides evidence of independent gene action for several possible mechanisms postulated for this trait *i.e.* pod wall thickness, elasticity of the wall material and the cross sectional shape of the pod valve. Expressing the results for the mean pod wall thickness and pod depth in a similar way (graph not shown) indicates that there is little dominance for these effects; thus additive gene action may be the major component in determining these results. Of these four characters, the slope of the regression in the hybrid/mid-parent relationship differs most from 1 (Fig. 4d) indicating the importance of SCA in these results. The position of the hybrid values indicates that genes within z79 and

dk142 confer greater pod compliance while those of dk150 confer greater rigidity. Elasticity of the cell wall was not calculated.

Pod, raceme and plant characters

Statistically there were significant block effects and family × block interactions for many of the pod, raceme and plant characters (Table 3, columns 2 - 4) though the values of the interaction mean squares were small compared to those for the main family effects and thus, of little biological importance. Total variance was apportioned largely between error variance (Φ^2) and among family variance (Φ^2_{family}) with little variation resulting from either block effects or family × block interactions (Table 3, columns 5 - 8). Despite their significance block effects accounted for less than 5% while the average family × block interaction was only 6%. of the variation for all characters. All characters showed highly significant variation among families (Φ^2_{family}) with the proportion accounted for rising from 17.3% for the number of primary branches to 61% for the number of seeds per pod.

In all cases but one, non-additive gene effects, as determined by the male × female interaction, were statistically highly significant (Table 4) though the mean squares were mostly much lower in magnitude than those of the combined male and female main effects (7% on average). Exceptions were observed for stem thickness and the number of primary branches where these values rose to 34% and 55% respectively. There was significant variation among both male and female arrays though the relative magnitude of this variation varied between male and female arrays; thus for example, males showed greater variation for plant height and females greater variation for pod length. Overall levels of combining ability were high (66 - 80%); however, stem thickness and the number of primary branches were very low (< 40%) with pod density and mean seed weight intermediate (*c* 55%).

DISCUSSION

The characters described in detail above (but also including peak load) are the main ones used in this study to define the pod shattering resistance trait and each measured a different aspect of the character. They thus contribute different information needed to interpret and understand why some pods are more shatter resistant than others and how these differences are regulated.

These analyses of the pod shattering resistance trait show that the various measures of assessing the phenotypic expression of the character give different indications of the gene actions involved and that there are, therefore, likely to be several different, independent genes involved. Overall, additivity (Table 4) is much greater than non-additive gene effects; thus the force needed to initiate pod dehiscence (peak load) and the energy needed to extend the initial fracture (fracture energy) have non-additive components contributing less than 15% of additive gene effects (Table 4). In contrast recovered energy is regulated to a greater extent by dominant gene action (66% of addititive effects). Recovered energy, a measure of stored energy, derives from the shape and deformation of the valve and was related to the mean pod wall thickness and also the depth of the pod (this was a measure of the degree of 'cuppedness' and was highly correlated with the ratio of pod width to depth). Thus line dk129, which had the most compliant pods where the pod walls were the most elastic (recovered energy, 0.66J), had the deepest and most thick walled pods (Table 2). Apex, with relatively thin walls and deep pods, and z79, with thicker walls and deep pods, showed less elasticity (0.34J and 0.28J respectively) while the remaining lines which generally had the thinnest walls and less deep pods

(mean = 0.17J) were stiffest. Field score for pod shattering resistance, which is a measure of the resultant actions of all the other traits, was intermediate to these characters (32% dominant gene effects) and appeared to behave as if resistance was controlled by recessive genes. In comparison, the number of pods remaining intact after 20s in the random impact tests, which is an attempt to devise a laboratory test under controlled conditions mimicking the situation in the field, behaves in a largely additive manner suggesting that the forces acting during tactile bending of the pods in the field differ from the random impacts that arise from the ball bearings in the random impact tests. Dehiscence in the field occurs naturally at the pedicel end of the pod and then extends to the beak while it was noted that in the random impact tests the beak was often broken first thus allowing the initiation of dehiscence from both ends of the pod.

Of the plant, raceme and pod characters measured, only plant height, pod wall thickness and pod depth showed important correlations with the several measures of pod shattering resistance. Those relationships with the most important biological significance are described in Fig. 1. There were extremely close associations between field score and peak load and fracture energy (tensile separation tests) which were, in turn, less well associated with the pod measurements of wall thickness and depth (shape). The association of these three measures of pod shattering resistance was slightly lower with the number of intact pods at 20s (random impact tests) which, however, showed no association with pod wall thickness and shape. This demonstrates that the tests are different in nature and measure different aspects of the resistance mechanisms. The energy recovered during tensile separation tests was strongly associated with the pod characters measured but only less well with fracture energy and peak load, again suggesting that pod architecture was only partly responsible for pod shattering resistance. There were many other statistically significant correlations which had low coefficients of determination (r^2) and were of little biological importance in these tests, though perhaps some, like pod angle and pod length, would be significant in the crop canopy. The one exception to this was plant height and to a lesser extent beak length as also described by Morgan et al. (1998). It is possible that these are genetically linked characters which have no direct bearing on the shatter resistance of these lines. The associations may reflect similar origins from the diploid parents used to make the synthetic oilseed rape.

When considering the potential of pod shattering resistance in crop improvement, other pod characters are likely to be important in addition to those described above, as also are aspects of the crop canopy within the field situation. Though these characters are likely to be secondary in importance to the primary pod structure, it is important to consider their phenotypic effects and genetic control. For example, erect pods might be directly beneficial, resulting in a canopy in which the pods are 'protected' from damage by their closeness to the rachis; however, short pods with thick walls might give stronger pods but confer serious penalties in yield resulting from an increase in the dry matter of the pod walls. These must be weighed against the negative effects of correlations such that deleterious pleiotropic effects must be eliminated from the genome. The extent to which these characters can be manipulated depends also on the strength of their genetic control as well as on gene linkage and pleiotropy. There are strong correlations among those characters directly measuring aspects of pod shattering resistance but these are not, or are only loosely, correlated with the other morphological characters which might be expected to have a bearing on resistance. This suggests that gene linkage or pleiotropy are not likely to restrict the success of a breeding programme. Prospects for successful incorporation of the shatter resistance character through a breeding

programme are enhanced by the strong heritabilities estimated for most of the characters. Within this diallel programme the degree of heritability was very high for most characters (Table 4) suggesting that it should be possible to combine and incorporate any of these characters into suitable genetic backgrounds for commercial purposes. However, introgressing such complex, recessive traits within a conventional breeding programme is difficult so the use of marker assisted technology within a breeding programme would be beneficial.

ACKNOWLEDGMENTS

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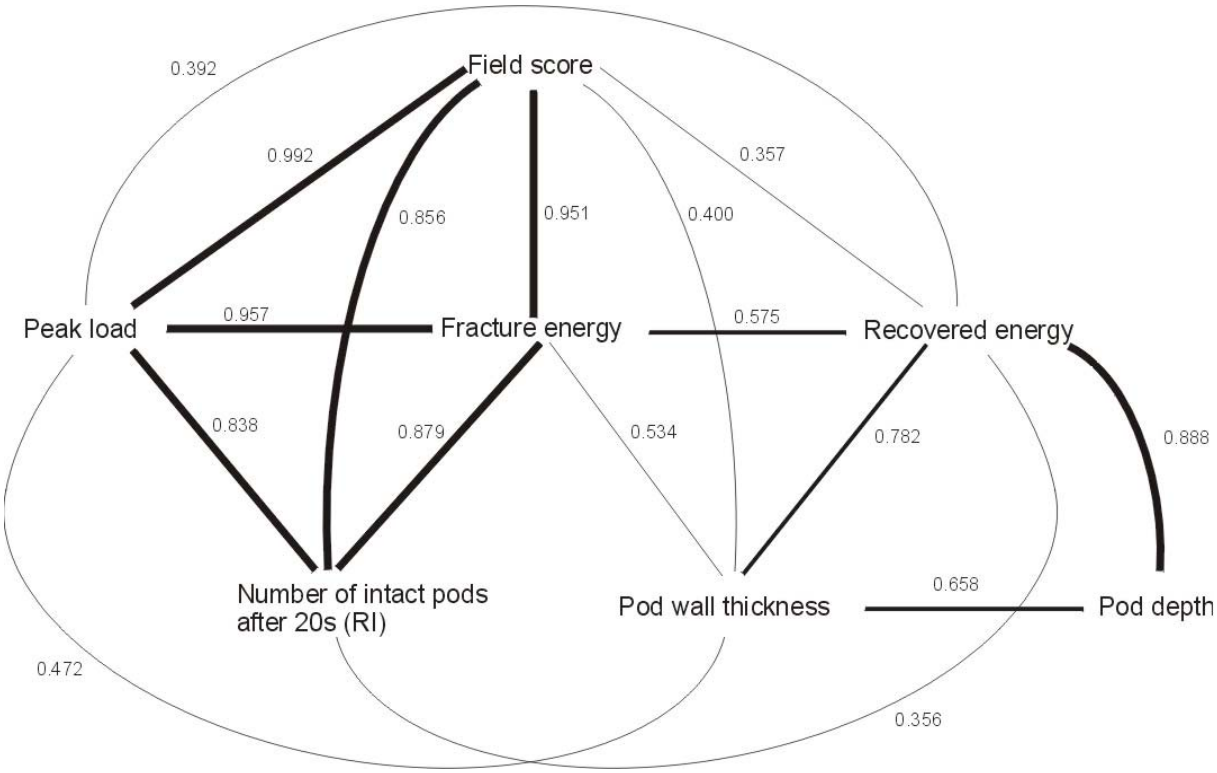
Fig. 1. Correlation coefficients among selected characters most related to pod shattering resistance.

Width of lines indicates value of coefficient of determination:

> 70%; 30 - 69%; < 30%;



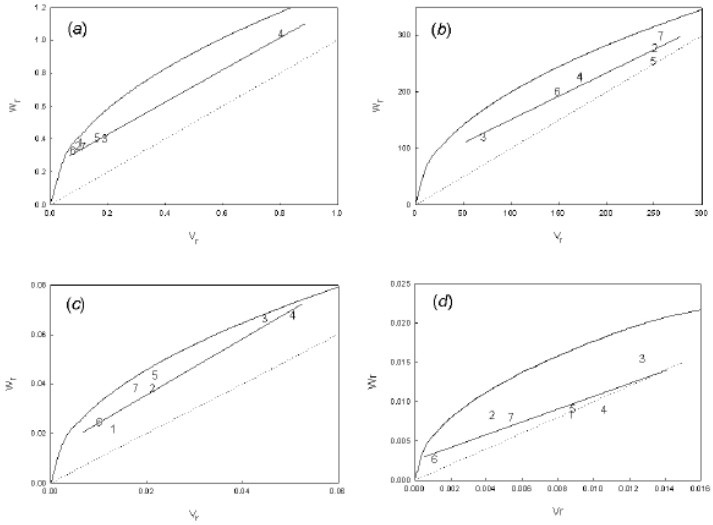


Fig. 2. Relationship between the variance of the offspring for each parental line (V_r) and their co-variance with the recurring parent (W_r). (*a*) Mean pod wall thickness; (*b*) Field pod shatter score; (*c*) Number of intact pods after 20 s random impact; (*d*) Pod fracture energy (tensile separation); The parabola defines the theoretical limit to the W_r:V_r ratio (calculated from $W_r^2 = V_r \times V_p$; where V_p is the variance of the parents). Points lying on the 1:1 line indicate full dominance for that character while points lying on the parabola indicate absence of dominance.
Lines are: 1. q28; 2. z79; 3. dk129; 4. dk142; 5. dk150; 6. Apex; 7. Tapidor.

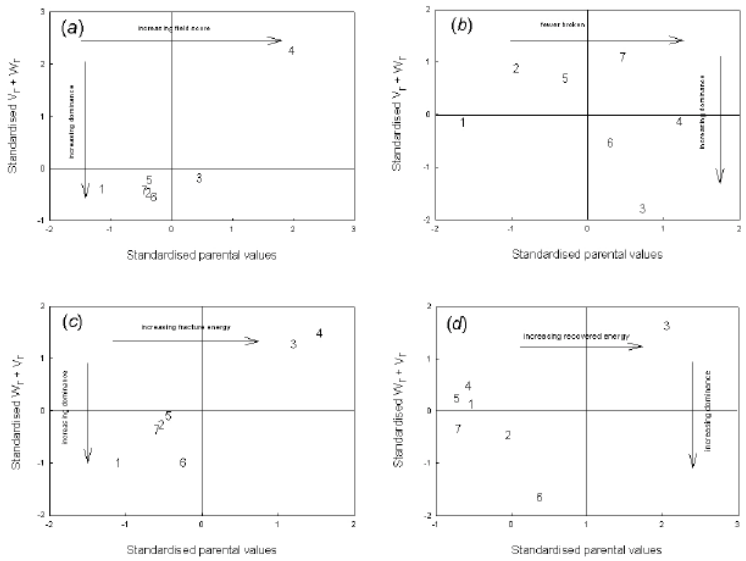


Fig. 3. Relationship of the phenotypic expression of a character to the extent of its genetic dominance. Points are standardised to the mean parental value using $(\bar{x} - x) / \sigma^2$. (*a*) Mean pod wall thickness; (*b*) Field pod shatter score; (*c*) Number of intact pods after 20 s random impact; (*d*) Pod fracture energy (tensile separation).
Lines are: 1. q28; 2. z79; 3. dk129; 4. dk142; 5. dk150; 6. Apex; 7. Tapidor.

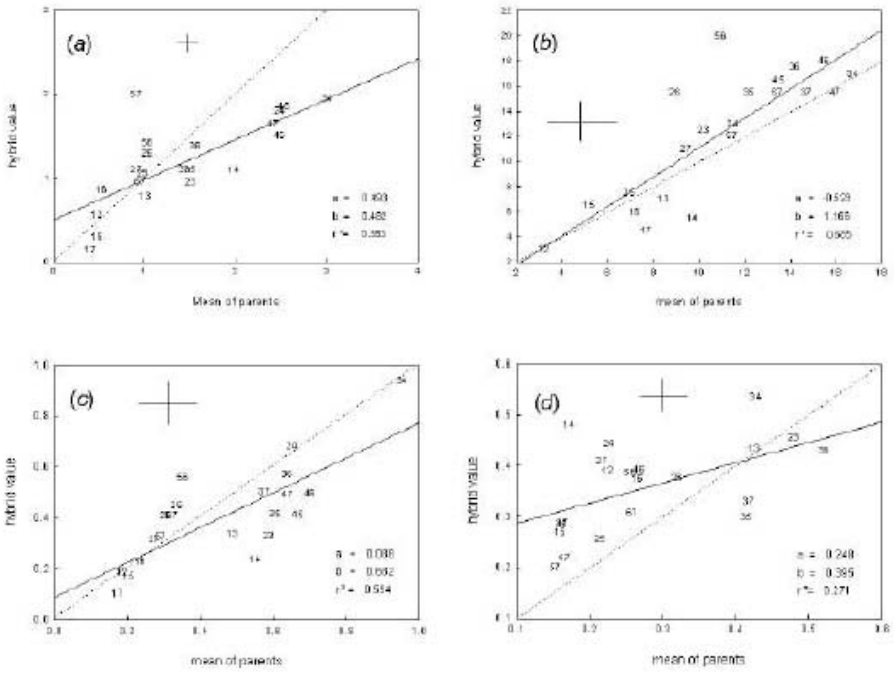


Fig. 4. Regression of family (hybrid) means and the mean value of their parents.
The slope of the solid line (b) indicates additivity of gene action. Dotted line is 1:1 slope.
Numbers indicate pairs of parents in reciprocal crosses.
1. Q28; 2. Z79; 3. DK129; 4. DK142; 5. DK150; 6. Apex; 7. Tapidor.
Bars indicate 2 x inter plant SE.

Table 1. *Material used in diallel analysis*

Line	Derivation of material	Expected resistance
q28[a]	Tapidor[b] × (*B. oleracea atlantica* × *B. rapa* '29')[c]	1
z79[a]	Tapidor[b] × (*B. oleracea macrocarpa* × *B. rapa* '29')[c]	1.5
dk129[a]	(*B. rapa chinensis* × *B. oleracea alboglabra*)[c] × N-O-109[d]	3
dk142[a]	N-O-109[d] × (*B. rapa chinensis* × *B. oleracea alboglabra*)[c]	4
dk150[a]	N-O-109[d] × (*B. rapa chinensis* × *B. oleracea alboglabra*)[c]	2
Apex	Cultivar	2
Tapidor	Cultivar	1.5

[a] Doubled haploid *B. napus* line
[b] Doubled haploid *B. napus* cultivar
[c] Synthetic *B. napus*
[d] *B. napus* breeding line

Table 2. *Parental means*

	q28	z79	dk129	dk142	dk150	Apex	Tapidor	SE[1]
Number of days to first flower (after 22 April)	4.0	23.3	12.8	12.7	14.4	20.4	18.9	0.96
Plant height (cm)	96.4	107.6	161.6	139.9	140.9	130.3	131.6	4.50
Stem thickness (cm)	11.5	10.7	15.3	19.2	18.5	15.6	16.9	1.22
Number of primary branches	5.8	5.0	5.5	6.2	6.5	7.6	5.4	0.42
Pod length (mm)	38.8	38.7	42.9	37.9	45.8	62.6	65.7	2.28
Beak length (mm)	18.6	10.8	9.9	14.6	11.0	14.4	14.4	0.61
Angle of pod to rachis (^{B})	36.4	61.4	29.7	27.1	32.1	68.9	68.3	2.83
Estimated raceme width (mm)	108.8	118.1	86.8	76.1	93.8	180.7	178.7	6.79
Pod density (per cm)	1.19	1.29	0.51	0.69	0.93	1.03	1.14	0.06
Number of seeds per pod	12.8	10.3	10.4	4.6	8.9	23.2	23.2	1.32
Mean seed weight (mg)	3.2	3.5	5.6	3.4	3.9	4.9	4.1	0.22
Depth of pods (mm)	4.2	4.8	6.2	3.9	3.6	4.6	4.2	0.14
Mean pod wall thickness (mm)	0.45	0.52	0.62	0.51	0.42	0.33	0.31	0.018
Field score for pod shattering index[2]	0.01	0.98	2.05	3.74	1.00	1.10	0.90	0.158
Number pods intact after 20 seconds	1.50	5.00	15.50	18.08	9.00	13.00	14.00	0.950
Peak load (N)	0.74	2.64	4.77	6.00	2.05	2.73	2.15	0.275
Recovered energy (J)	0.18	0.28	0.66	0.20	0.15	0.34	0.16	0.031
Fracture energy (J)	0.09	0.29	0.88	1.04	0.32	0.38	0.27	0.054

[1] SEs determined from Error MS derived from male × female anova with 392 df

[2] 0 = shatter susceptible; 4 = shatter resistant

Table 3. *Analysis of variance for all characters*

Character	Mean squares from anova				Component of variation %			
	Between families (df = 48)	Between blocks (df = 1)	Families × Blocks (df = 48)	Error mean square (df = 392)	(Φ^2_{family})	(Φ^2_{block})	($\Phi^2_{family.block}$)	(Φ^2)
Number of days to first flower (after 22 April)	122.64(((	23.00	16.59(((	8.592	51.0	0	7.7	41.3
Maximum plant height	1780.3(((	2667.0(((	151.9	194.8	45.0	1.3	0	53.8
Stem thickness	68.33(((	336.48(((	20.47	16.26	20.9	4.3	3.7	71.1
Number of primary branches	5.316(((	14.253((	1.763	1.636	17.3	1.9	1.2	79.6
Pod length	759.50 (((	596.32(((	77.68(((	42.82	57.0	1.4	5.8	35.8
Beak length	48.479(((	0.128	4.214	3.367	55.6	0	2.1	52.3
Angle of pod to rachis	1281.58(((	2.09	81.22	82.61	59.2	0	0	40.8
Estimated raceme width	7175.7(((	1589.5	520.7	454.8	58.5	0.3	0.1	40.0
Pod density	0.200(((	0.051	0.046(	0.032	31.1	0	5.7	63.3
Number of seeds per pod	307.89(((	184.78(((	26.24((	15.52	60.8	1.1	4.6	33.5
Mean seed weight	2.524(((	0.238	0.720(((	0.395	28.2	0	10.1	61.7
Depth of pods	1.931(((	0.003	0.323(((	0.148	46.8	0	10.2	43.0
Mean pod wall thickness	5.543(((	0.263	0.512	0.319	42.8	0		
Field score for pod shattering index	3.390(((	9.405(((	0.290	0.258	51.4	4.7	1.1	42.8
Number pods intact after 20 seconds	54.52(((	-	-	9.92[1]	69.2	-	-	30.8
Peak load	10.811(((	0.247	1.636	0.620	52.7	0	11.7	35.6
Fracture energy	0.404(((	0.139(	0.047((	0.027	52.9	0.4	6.2	39.8
Recovered energy	0.097(((	0.216(((	0.031(((	0.006	35.6	3.1	26.4	34.8

except [1] where Error MS = 49 df

Table 4. *Analysis of variance for all characters*

Character	Mean squares from anova				Component of variation %			
	Between males (df = 6)	Between females (df =6)	males × females (df = 36)	Error mean square (df = 392)	(Φ^2_{males})	($\Phi^2_{females}$)	($\Phi^2_{males.females}$)	(Φ^2)
Number of days to first flower	387.89	584.75	32.26	9.19	25.6	39.7	11.6	23.1
Plant height	7466.6	3616.4	888.7	202.5	31.0	12.9	22.7	33.4
Stem thickness	166.44	61.20	39.54	14.81	14.7	2.5	20.1	60.2
Number of primary branches	8.228	8.870	4.704	1.737	4.0	4.7	23.3	68.1
Pod length	1377.21	2886.43	304.39	51.94	14.8	35.7	24.4	25.1
Beak length	183.83	181.32	14.78	3.66	31.2	30.8	14.4	23.7
Angle of pod to rachis	4486.98	5074.41	344.03	79.92	30.6	35.0	13.7	20.7
Estimated raceme width	21755.2	33589.5	1958.3	460.6	25.4	40.5	13.4	20.7
Pod density	0.571	0.377	0.178	0.035	27.8	17.4	9.6	45.2
Number of seeds per pod	648.03	1228.46	91.44	17.26	19.8	40.4	18.4	21.4
Mean seed weight	6.219	9.944	1.393	0.482	13.2	23.3	17.4	46.1
Depth of pods (mean of pods)	5.475	8.592	0.626	0.186	21.6	35.5	13.7	29.1
Mean pod wall thickness ($\times 10^3$)	2.122	2.112	0.100	0.034	35.7	35.5	8.3	20.6
Field score for pod shattering index	12.278	11.310	1.231	0.249[1]	30.1	27.5	18.7	23.7
Number pods intact after 20 seconds	199.15	174.34	12.64[ns]	9.03	42.7	37.0	5.8	14.5
Peak load	36.156	52.120	2.853	0.755	26.9	39.8	11.9	21.4
Fracture energy	1.4052	1.9342	0.0918	0.0288	28.5	40.0	9.6	21.9
Recovered energy	0.2339	0.3578	0.0549	0.0095	15.8	26.7	28.0	29.4

n = 5; except[1] where Error MS = 49 df; all MS are significant to $p < 0.001$ except where indicated

Table 5. *Summary of 7 x 7 diallel data from selected characters*

Character	Additivity ('a' MS from Hayman ANOVA)[1]	Dominance ('b' MS from Hayman ANOVA)				D	H1	H2	Ratio of dominance : additivity /(H_1/D)
		b[2]	b1[3]	b2[1]	b3[4]				
Field shatter score	3.768***	0.257***	0.996***	0.634***	0.043^{ns}	1.598	0.533	0.271	0.58
Number of intact pods at 20s (RI)	204.3***	10.33*	7.292^{ns}	18.89**	6.88^{ns}	30.22	11.15	6.378	0.61
Fracture energy (TS) ($\times 10^4$)	0.427***	0.0151**	0.030*	0.024**	0.01*	0.117	0.0226	0.015	0.44
Recovered energy (TS)	0.082***	0.0127***	0.062***	0.025***	0.004*	0.033	0.019	0.011	0.77

[1] df = 6; [2] df = 21; [3] df = 1; [4] df = 14

D = additive genetic variation

H1 = dominance

H2 = assymetrical distribution

All MSs tested against block interactions from Hayman ANOVA. When tested against the Error MS for the Family analysis (table 3) the variance ratios were of similar magnitude but were significant more often because of the much greater degrees of freedom associated with the Family Error MS.