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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.

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MATERIALS 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 vernalizing for 8 weeks at 6 °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 vernalized as above and transplanted into the field in March 1998. Plants were grown at a spacing of 0·5 m within rows which were 1 m apart in two fully randomized 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. 22 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 five 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. 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

Table 1. *Material used in diallel analysis*

Line	Derivation of material	Expected resistance
q28*	Tapidor† × (*B. oleracea atlantica* × *B. rapa* '29')‡	1
z79*	Tapidor† × (*B. oleracea macrocarpa* × *B. rapa* '29')‡	1·5
dk129*	(*B. rapa chinensis* × *B. oleracea alboglabra*)‡ × N-O-109 §	3
dk142*	N-O-109 § × (*B. rapa chinensis* × *B. oleracea alboglabra*)‡	4
dk150*	N-O-109 § × (*B. rapa chinensis* × *B. oleracea alboglabra*)‡	2
Apex	Cultivar	2
Tapidor	Cultivar	1·5

* Doubled haploid *B. napus* line.
† Doubled haploid *B. napus* cultivar.
‡ Synthetic *B. napus*.
§ *B. napus* breeding line.

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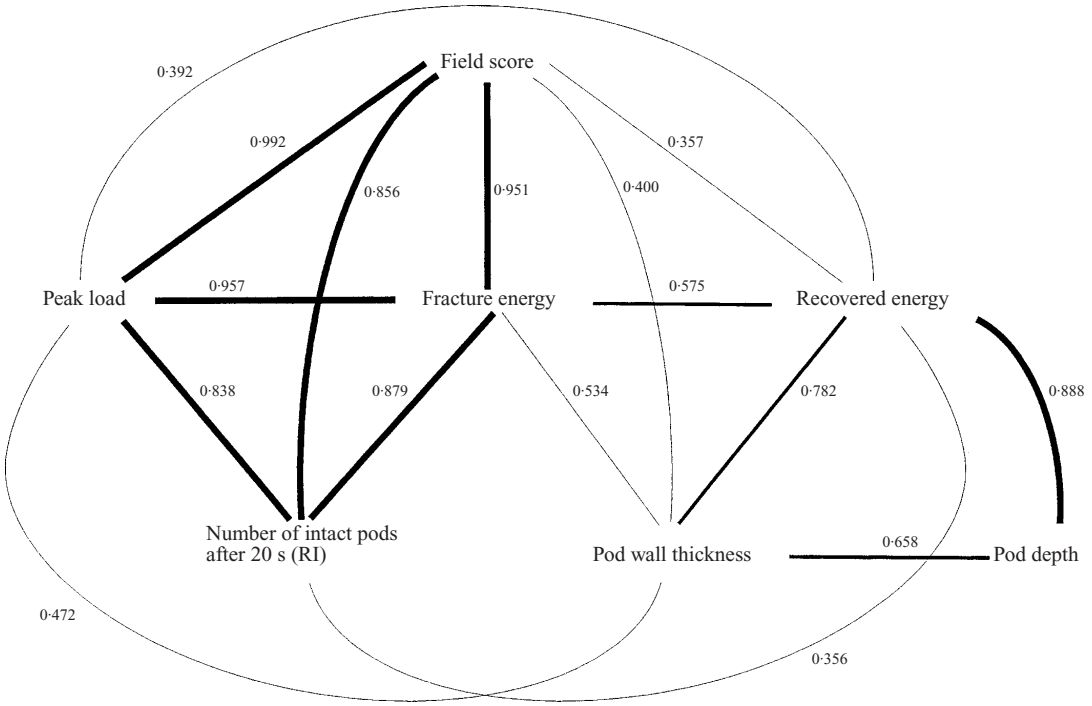

Fig. 1. Diagram illustrating the relationships among selected characters associated with pod shattering resistance as determined by their correlation coefficients (D.F. = 47). All are significant at $P < 0{\cdot}001$. Width of lines indicates value of coefficient of determination (r^2): ▬ > 70 %; — 30–69 %; ── < 30 %.

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 characterizes 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 20 s of standardized shaking in a drum with ball bearings was counted. This 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 (D.F. = 48) and blocks (D.F. = 1) as main effects tested against the amongst replicate plant error (D.F. = 392). Subsequent analyses were carried out to estimate male and female effects (D.F. = 6) and male × female interaction (D.F. = 35) for which the error term included all other interactions (i.e. blocks × males, blocks × females and blocks × males × females; D.F. = 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 σ^2_g ($\sigma^2_{male} + \sigma^2_{female} + \sigma^2_{male.female}$) were highly correlated with σ^2_{family} obtained from the analysis of

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family × block ($b = +0{\cdot}916$; $r = +0{\cdot}931$) but were, on average 31% higher. These apparently higher values of σ_g^2 resulted from the removal of block effects and interactions from the error term in the second analysis. The values of σ_g^2 derived from the male × female analyses were subdivided into $\sigma^2_{male} + \sigma^2_{female}$ and $\sigma^2_{male.female}$ which were equated with general combining ability (GCA) and specific combining abilities (SCA) respectively.

Five of the traits used to characterize 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 combining 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 5 (Genstat 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 characterized 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 incompatibility 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

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Table 2. *Parental means*

	q28	z79	dk129	dk142	dk150	Apex	Tapidor	S.E.*
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 (°)	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†	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

* S.E.S determined from error MS derived from male × female ANOVA with 392 D.F.
† 0, shatter susceptible; 4, shatter resistant.

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 significant family and block effects (Table 3) though when σ^2_{block} was estimated as a percentage, its contribution to the total variation was only 4%. 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 ($\sigma^2_{\text{male}} + \sigma^2_{\text{female}}$) were three times greater than the non-additive component ($\sigma^2_{\text{male.female}}$) 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) ($\sqrt{(H_1/D)}$) 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. 2*a*). The regression line did not differ significantly from 1 and lies midway between the 1:1 line and the parabola suggesting the presence of 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. 2*a* in which the standardized total mean squares of the array variance and covariance are plotted against the standardized pod shatter scores (data were standardized as $(\bar{x}-x)/\sigma^2$. 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. 4*a* 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

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Table 3. *Analysis of variance for all characters*

Character	Mean squares from ANOVA: Between families (D.F. = 48)	Between blocks (D.F. = 1)	Families × Blocks (D.F. = 48)	Error mean square (D.F. = 392)	Component of variation % (σ^2_{family})	(σ^2_{block})	($\sigma^2_{family.block}$)	(σ^2)
Number of days to first flower (after 22 April)	138·7***	15·3	19·5***	8·743	52·3	0	9·4	38·3
Maximum plant height	2056·4***	2864·7***	153·4	213·6	45·1	2·6	0	52·3
Stem thickness	54·1***	240·5***	22·0	14·4	16·1	4·5	7·6	71·9
Number of primary branches	5·51***	13·01**	2·01	1·74	16·0	2·1	2·5	79·4
Pod length	778·7***	508·8***	94·0***	49·5	53·3	1·3	6·9	38·5
Beak length	56·56***	1·37	4·14	3·73	57·9	0	0·9	41·2
Angle of pod to rachis	1497·1***	101·6	89·7	80·9	63·0	0	0·8	36·2
Estimated raceme width	8548·9***	441·2	608·7	455·1	62·0	0	2·4	35·6
Pod density	0·232***	0·041	0·056*	0·036	30·5	0	6·9	62·6
Number of seeds per pod	305·0***	115·7***	31·4***	16·3	58·2	0·7	6·4	34·7
Mean seed weight	3·10***	0·40	0·84**	0·46	29·6	0	10·2	60·2
Depth of pods	2·24***	0	0·39***	0·17	46·1	0	11·0	42·9
Mean pod wall thickness	6·08***	0·53	0·50	0·33	60·6	0	3·7	35·7
Field score for pod shattering index	3·87***	6·25***	0·32	0·25	55·4	3·8	2·3	38·5
Number pods intact after 20 seconds	55·35***	—	—	8·84†	72·5	—	—	27·5
Peak load	12·88***	0·44	1·76	0·65	56·1	0	11·3	32·6
Fracture energy	0·477***	0·162*	0·038**	0·028	53·5	0·6	11·4	34·5
Recovered energy	0·113***	0·201***	0·034***	0·007	37·9	3·3	25·6	33·2

† Error MS = 49 D.F. ***, $P < 0{\cdot}001$; **, $P = 0{\cdot}01$–$0{\cdot}001$; *, $P = 0{\cdot}05$–$0{\cdot}01$.

Table 4. *Analysis of variance for all characters*

Character	Mean squares from ANOVA: Between males (D.F. = 6)	Between females (D.F. = 6)	Males × Females (D.F. = 36)	Error mean square (D.F. = 392)	Component of variation %: (σ^2_{males})	($\sigma^2_{females}$)	($\sigma^2_{males.females}$)	(σ^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†	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

† Error MS = 49 D.F.; all MS are significant to $P < 0{\cdot}001$ except where indicated.

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

Character	Additivity ('a' MS from Hayman ANOVA)	Dominance ('b' MS from Hayman ANOVA)				D	H1	H2	Ratio of dominance: additivity $\sqrt{(H_1/D)}$
		b_1‡	b_1§	b_2†	b_3††				
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 20 s (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

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.
†, D.F. = 6; ‡, D.F. = 21; §, D.F. = 1; ††, D.F. = 14.
***, $P < 0{\cdot}001$; **, $P = 0{\cdot}01$–$0{\cdot}001$; *, $P = 0{\cdot}05$–$0{\cdot}01$.
D, additive genetic variation; H1, dominance; H2, assymetrical distribution.

analysis or $\sigma^2_{male} + \sigma^2_{female}$ 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 ($\sigma^2_{male} + \sigma^2_{female}$) were very high (80%) in contrast to non-additive gene effects ($\sigma^2_{male.female}$) which were very low (6%). As with field shatter score, the genetic component (σ^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. 3*b*. A slope of unity in Fig. 4*b* 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 (Table 3), and to a lesser extent block effects. However, as before, these effects were small compared to the family effect and error; thus σ^2_{family} accounted for 54% of the variation while $\sigma^2_{family.block}$ was only 11%. Variation amongst male and female arrays was again very large with no male × female interaction. Total variation due to genetic factors (σ^2_g) was very high (69%) with a contribution of 10% from dominant gene effects ($\sigma^2_{male.female}$) to σ^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. 2*c*. 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. 3*c*). When the hybrid values were plotted against the mean of their parents (Fig. 4*c*) the fitted regression showed a

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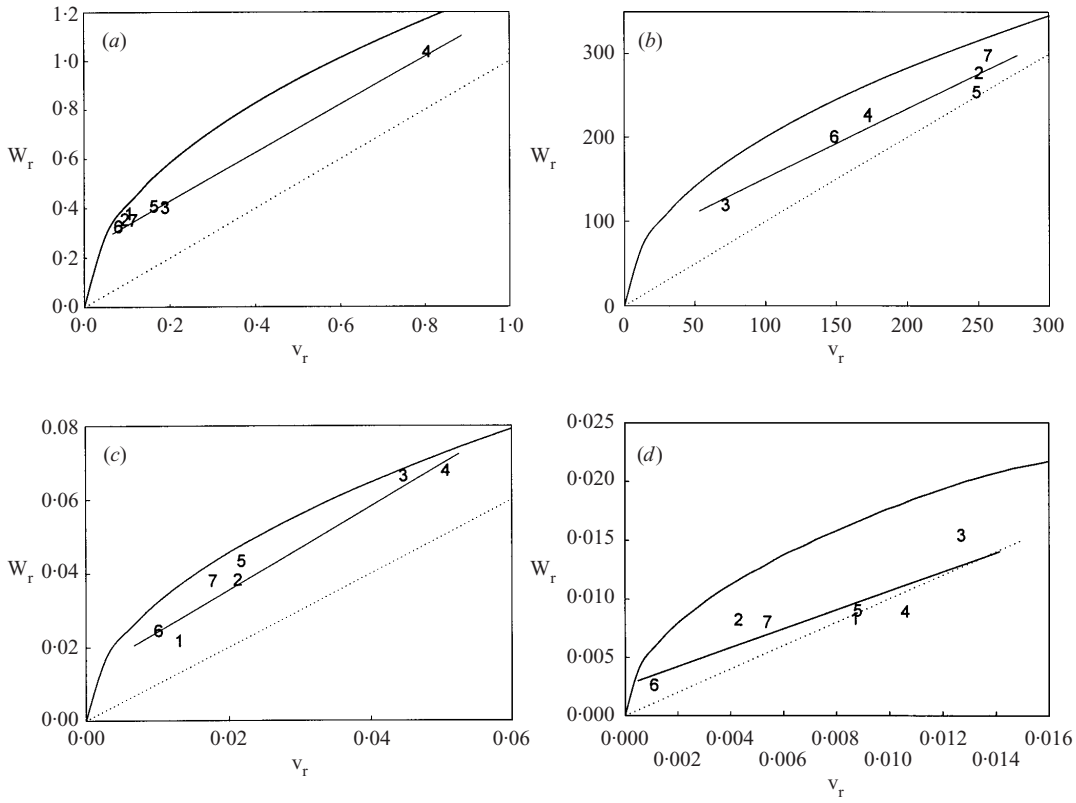

Fig. 2. Relationship between the variance of the offspring for each parental line (V_r) and their covariance with the recurring parent (W_r). (*a*) Mean pod wall thickness; (*b*) field pod shatter score; (*c*) number of intact pods after 20 s random impact; (*d*) pod fracture energy (tensile separation). 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.

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 38% 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 ($\sigma^2_{\text{male.female}}$) compared to 44% for additive effects ($\sigma^2_{\text{male}} + \sigma^2_{\text{female}}$). 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. 2*d* also indicates the presence of dominance gene effects. Plotting the genotypic against the phenotypic expression of this character shown in Fig. 3*d* 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

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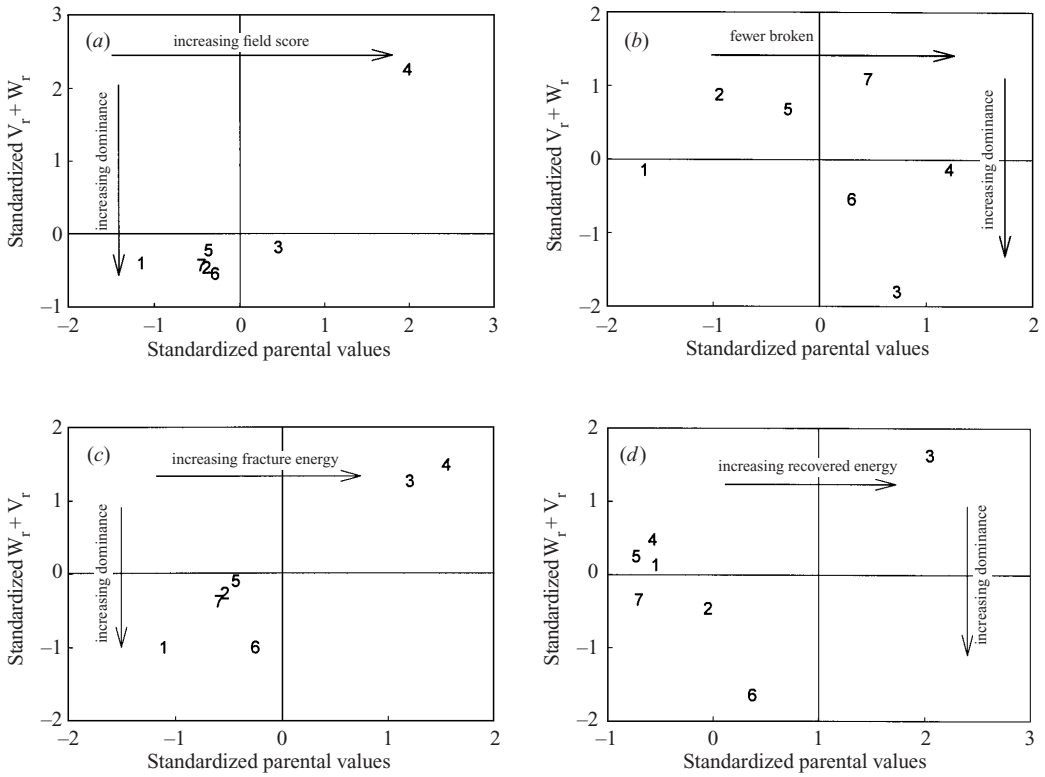

Fig. 3. Relationship of the phenotypic expression of a character to the extent of its genetic dominance. Points are standardized to the mean parental value using $(\bar{x} - x)/\sigma^2$. (*a*) Mean pod wall thickness; (*b*) field pod shatter score; (*c*) number of intact pods after 20 s random impact;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

regression in the hybrid/mid-parent relationship differs most from 1 (Fig. 4*d*) 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 also small compared to those for the main family effects with a mean value of only 4% and thus, of little biological importance. Total variance was apportioned largely between error variance (σ^2) and among family variance (σ^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 (σ^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%).

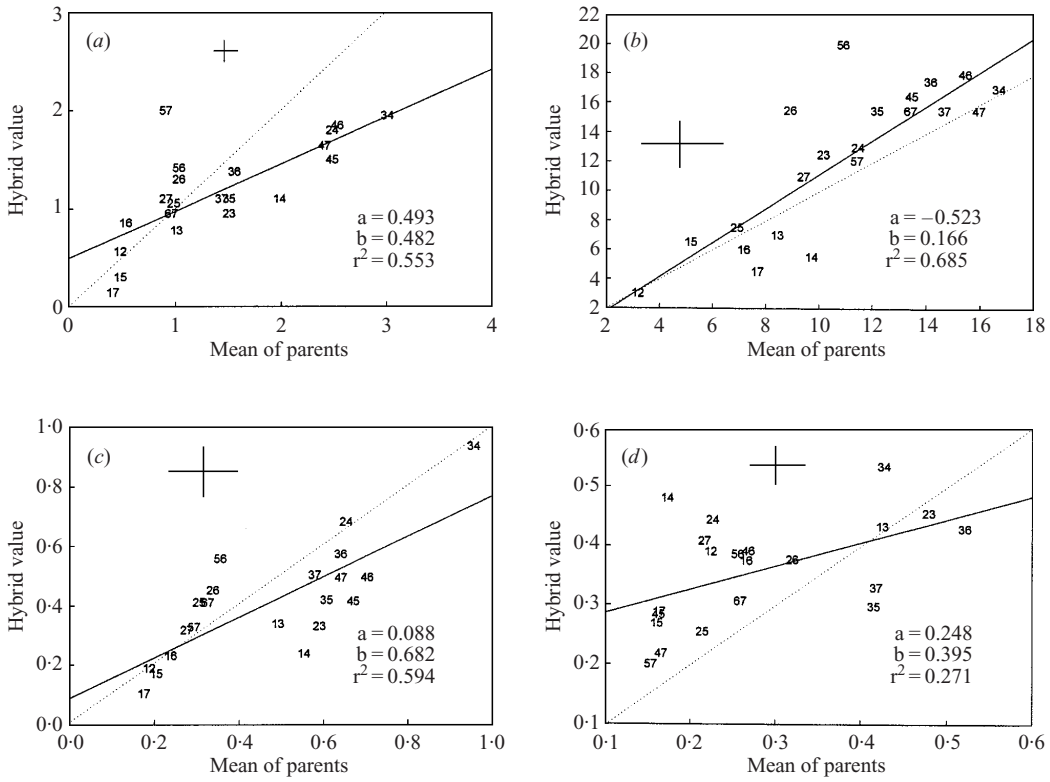

Fig. 4. Relationship between family (hybrid) means and the mean value of their parents. The slope of the line (b) indicates additivity of gene action such that 1 = full additive gene action and 0 = full dominance gene action. First number of each point is female parent; second number is male parent. Parents are: 1. q28; 2. z79; 3. dk129; 4. dk142; 5. dk150; 6. Apex; 7. Tapidor.

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, 0·66 J), 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 (0·34 J and 0·28 J respectively) while the remaining lines which generally had the thinnest walls and less deep pods (mean = 0·17 J) 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 20 s in the random impact tests, which is an attempt to devise a laboratory test under controlled conditions mimicking

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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 20 s (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.

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