The potential of selected *Linum usitatissimum* crosses for producing recombinant inbred lines with dual-purpose characteristics

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SUMMARY

The success of *Linum usitatissimum* as a commercial crop depends on its value for seed/oil (linseed) and/or fibre/straw production (flax). In the present study we evaluated nine linseed × linseed and two linseed × flax crosses for their potential to produce recombinant inbred lines (RILs) that give higher yields for dual-purpose (high seed–high fibre) traits. Analysis of the early generations indicated that while all crosses segregated for seed weight, straw weight, capsule weight and total plant weight, some crosses lacked the necessary genetic diversity to produce superior RILs. The performance of F_3 families supported this conclusion as only two crosses were identified which had adequate potential to produce improved RILs with high seed and straw/fibre yields. The four most heterotic crosses identified in a previous experiment showed little potential for transgressive recombinants, although the best of these RILs would make better dual-purpose varieties compared to existing cultivars. Genotypic correlations were generally positive and should increase the chances of extracting RILs producing both high seed/oil and straw/fibre yields.

INTRODUCTION

Linum usitatissimum is grown commercially for two products, seed and fibre. Usually separate cultivars are used to obtain these products and they are called seed flax or linseed, when grown for seed production, and fibre flax or flax, when raised for fibre. Linseed oil has many industrial applications and the seed cake is used as animal feed while flax fibre is used to produce high quality linen. The European Union (EU) exports raw and processed flax fibre to many developing and developed countries while large quantities of linseed seed are imported at a cost of millions of euros (Gilbertson 1990). Thus, cultivation of linseed will not only provide valuable income to farmers but also reduce EU imports.

The commercial worth of linseed, which is a relatively minor crop in the EU and UK, can be improved by using it in a dual-purpose capacity. While the primary product of the linseed crop is its seed and oil, the low grade and low quantity of the fibre/straw it produces can be utilized for making new industrial products such as fibre matting, pollution

filters and automobile body parts (Kessler & Tubach 1995; Foster 1998). Foster *et al.* (1997) evaluated the existing linseed/flax varieties with this objective in mind but found that none of the varieties came even close to meeting a 'dual-purpose' criterion. Furthermore, some of the F_1 crosses that met a 'dual-purpose' criterion could not be used directly as commercial varieties because it was not possible to produce hybrid seed on a commercial scale (Foster *et al.* 1998). Therefore, it is imperative that pure lines possessing dual-purpose characteristics can be developed for use as varieties. The present study assesses 11 F_1 crosses with the objective of identifying the most potent cross or crosses for producing pure breeding lines that will give high seed/oil cum high straw/fibre yields.

MATERIALS AND METHODS

The material used in this study consisted of 25 randomly produced F_3 families from each of 11 F_1 crosses that were selected on the basis of their desirable performance in an earlier trial (Foster *et al.* 1998). These crosses were A3 × B3, B3 × L2, B3 × N1, B3 × P2, L2 × N2, L2 × P1, A1 × B3, A1 × N2, B3 × N2, K2 × B3 and P1 × P2, and they included

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Table 1. *List of traits scored on the trial*

Symbol	Description
H1	Height in cm four weeks after sowing
Br	Indicator if a plant is branching after four weeks of sowing
FT	Number of days taken from sowing to first flowering
HFT	Height in cm at the time of flowering
CMT	Number of days to capsule maturity from sowing
H2	Height in cm after 7 weeks
HMT	Height in cm at the browning of the first capsule
TWt	Total weight in g of plant material from individual plots
NBr	Number of main branches per plant
StWt	Straw weight in g per plant
SdWt	Seed weight in g per plant

four F_1s (A3 × B3, B3 × L2, A1 × B3 and K2 × B3) that showed the best performance in terms of dual-purpose traits (see Foster *et al.* 1998 for codes of the parents). The 275 F_3 families, 11 original F_1 crosses and their selfs (F_2 families) and nine parental lines (A1, A3, B3, K2, L2, N1, N2, P1 and P2) were assessed in a randomized trial during the summer of 1996. Each family was represented in the trial by two single-row plots containing ten (2 × 5) plants in total. The experiment was divided into two blocks for convenience, the first block included the F_1, F_2 and F_3 generations of crosses 1–6 and the second accommodated the rest. The nine inbred parents were raised in both blocks and served as standards for making comparisons across the blocks.

The experiment was sown in the glasshouse to ensure good germination and one-week-old seedlings were transplanted in the field in rows 75 cm apart at a plant to plant distance of 15 cm. All experimental plants were scored for the 12 morphological traits listed in Table 1. The analyses of the F_1 and F_2 generations followed the nested design of Snedecor & Cochran (1989) where the between crosses mean squares (MS) were tested against the plot MS if the latter were significant when tested against within MS. The same procedure was applied to test differences between the F_3 families of each cross separately, and obtain estimates of the within families (σ_w^2), between families (σ_b^2) and between plots (σ_{plot}^2) components of variation. These components were later used to estimate heritability as $\sigma_b^2/(\sigma_b^2 + \sigma_{plot}^2 + \sigma_w^2)$ and predict the proportion of recombinant inbred lines scoring better than a chosen standard ($\bar{x}$) as a standardized normal integral from abscissa 'a' to ∞ where:

$$a = \frac{(\bar{x} - \bar{F}_3)}{\sqrt{(2 \times \sigma_b^2)}}$$

(see Kearsey & Pooni 1996 for further details).

The predictions and heritability were also calculated for the combined analysis of all the F_3 families (given in Table 3).

RESULTS AND INTERPRETATION

Average performance of the parents and crosses

The two way analysis of variance of the parental data indicated that block effects were significant for several traits, including StWt and SdWt (results not shown). In general, the parental lines performed significantly better in block 2, particularly for the traits scored at the end of the season. While this may have some consequences for the combined analysis of the two blocks, it is however not expected to cause any serious complications because the lines × blocks interaction was not significant. Comparison of the parental and F_1 means (Table 2) revealed that the hybrids generally grew more slowly, initially, and flowered later (on average 2·3 days later). However, plants that flowered 2 + days later tended to be taller and more productive (increased plant weight, seed weight and straw weight etc). The F_3 generations showed some inbreeding depression for many traits but its magnitude was low. This suggested that it should be possible to extract recombinant inbred lines that perform better than most, if not all, the F_1 hybrids. The parental and F_1 means of individual crosses also indicated that 'better parent' heterosis increased in magnitude two to threefold towards the higher end of the scale, particularly for TWt, CpWt, StWt and SdWt. However, a wider range of performance among the F_3 families also indicated that exploitable levels of genetic variation existed in many crosses.

Gene action/interaction

Scaling tests ($3\bar{F}_2 - 2\bar{F}_3 - \bar{F}_1$) and ($4\bar{F}_3 - \bar{F}_1 - 1\cdot5(\bar{P}_1 + \bar{P}_2)$) revealed that epistasis contributed very little to variation in any cross (results not shown). The F_3 families differed significantly for most traits, both within and between crosses (Table 3), but average heritability was generally low, except for plant height H2 and flowering time FT (Table 4). Apparently the genetic variance is low in most crosses and this could be due to a narrow genetic base for the parental lines

Table 2. *Comparative performances of the parental, F_1 and F_3 generations*

Family	H1*	Br	FT	HFT	H2	CMT	HMT	TWt	NBr	CPWt	StWt	SdWt
						Average performance						
Parents	14·9	2·0	20·0	68·3	43·1	33·1	87·7	191·7	6·0	69·6	122·2	38·2
F_1s	12·6	1·7	22·3	73·2	36·5	34·5	93·6	205·0	6·7	76·9	128·0	45·4
F_3s	13·5	1·8	21·8	68·9	38·2	33·8	86·9	194·6	6·7	66·9	117·4	39·8
						Highest mean						
Parents	17·5	2·3	25·5	86·4	54·9	37·3	108·2	245·0	7·6	88·4	157·0	48·6
F_1s	15·0	1·9	26·6	90·0	47·3	38·9	110·4	278·0	8·3	113·7	168·0	68·0
						Lowest mean						
Parents	13·1	1·0	13·7	55·0	37·0	26·1	79·3	108·5	4·5	34·5	81·5	23·0
F_1s	10·6	1·5	16·0	58·4	30·9	28·7	80·4	126·0	5·3	45·8	80·3	26·0

* See Table 1 and text for symbols.

Table 3. *Analysis of variance of the F_3 families*

Traits	Between crosses (10 df)	Families/crosses (264 df)	Plots/families (275 df)	Within plots (2200 df)
		Mean squares		
H1*	**141·11**†	**35·56**	**9·54**	4·96
Br	**10·69**	**2·05**	**1·13**	0·42
FT	**1723·82**	**79·60**	12·93	12·43
HFT	**9861·42**	**270·95**	**96·83**	66·71
H2	**4085·20**	**367·72**	**60·66**	37·05
CMT	**2118·56**	**72·36**	**40·89**	12·59
HMT	**5465·00**	**226·18**	**144·91**	80·85
TWt	**64517·20**	**5022·86**	2806·37	—
NBr	**20·50**	**3·39**	2·71	—
CpWt	**5119·70**	**858·12**	524·64	—
StWt	**36267·30**	**2453·08**	1418·02	—
SdWt	**621·80**	**247·09**	201·09	—

* See Table 1 and text for symbols.
† Values in bold are significant at $P \leqslant 0.05$.

Table 4. *The highest, the lowest and the combined values of heritability for traits and crosses*

Heritability	H1*	Br	FT	HFT	H2	CMT	HMT	TWt	NBr	CpWt	StWt	SdWt
						Traits						
Highest	0·54	0·36	0·58	0·71	0·67	0·42	0·24	0·47	0·35	0·43	0·51	0·31
Lowest	0·17	0·13	0·06	0·03	0·09	0·06	0·02	0·00	0·00	0·03	0·00	0·00
Combined	0·31	0·14	0·35	0·19	0·42	0·15	0·08	0·28	0·11	0·24	0·27	0·11

Heritability	1	2	3	4	5	6	7	8	9	10	11
						Crosses					
Highest	0·54	0·48	0·67	0·61	0·71	0·41	0·52	0·48	0·51	0·55	0·27
Lowest	0·11	0·07	0·07	0·00	0·00	0·00	0·00	0·00	0·13	0·07	0·03

* See Table 1 and text for meanings of the symbols.

Table 5. *Number of crosses (out of 11) with F_3 families showing transgression from the best of the parental or F_1 scores*

						Traits						
	H1*	BR	FT	HFT	H2	CMT	HMT	TWt	NBr	CpWt	StWt	SdWt
						Transgression for high score						
Crosses	6	10	8	1	4	4	0	4	7	3	9	3
						Transgression for low score						
Crosses	8	5	3	5	9	4	6	7	9	7	8	9

* See Table 1 and text for symbols.

Table 6. *Number of F_3 families that show transgression from the highest scoring parents for seed or/and straw weights (SdWt and StWt)*

		Crosses										
Trait	Category	1*	2	3	4	5	6	7	8	9	10	11
SdWt	$\geqslant P_{largest}$	4	3	7	6	3	1	12	3	6	9	10
StWt	$\geqslant P_{largest}$	5	9	2	15	12	9	4	0	11	2	1
Combined	$\geqslant P_{largest \,\&}$ $\geqslant P_{largest}$	2	2	1	5	2	0	2	0	4	1	0

* Cross 1 = A3 × B3, … Cross 11 = P1 × P2 etc, see materials and methods and Table 1 for symbols.

arising from the use of several closely related lines. However, a wide range of heritability values indicates that some crosses produced large genetic variances and therefore possessed good potential for producing useful recombinants.

Transgression among the F_3 families

The number of crosses in which F_3 families displayed transgressive segregation compared to the highest and lowest scores among the parental and F_1 families varied considerably among traits (Table 5). For instance, none of the crosses had an F_3 family that was taller than the tallest parent/F_1 at the time of maturity while 10 crosses had families with more branches compared to the most branched parent or F_1. Straw and seed weights also differed in this respect. Nine out of 11 crosses had F_3 families with a higher straw weight (StWt) than the best F_1 while only three crosses were higher for SdWt.

Transgression rates (Table 6) also indicated that crosses B3 × P2 and B3 × N2 had a high potential for producing superior RILs for SdWt and StWt simultaneously while high proportions of RILs from crosses A1 × B3, P1 × P2 and K2 × B3, were likely to have high seed yields and a moderate straw yield. Many F_3 families of crosses B3 × P2, L2 × N2, P1 × P2 and B3 × L2, on the other hand, showed promising performance for straw weight but they lacked potential for high seed yield.

The highest and lowest scores among the F_3 families of the four most heterotic crosses generally fell short of the best scores among all the F_3 families (see Table 7). Furthermore, these scores were much lower than the best F_3 scores for seed and straw yields. Thus, it was clear that the inbreeding in these crosses would not yield superior RILs with dual-purpose characteristics.

Correlations and predictions

Genetic correlations indicate potential for simultaneous improvement of traits during selection. Positive correlations enhance the improvement of traits in the same direction while negative correlations restrict simultaneous improvement. In the present study, correlations of the F_3 families showed that associations between TWt and StWt, and TWt and CpWt were very strong (see Table 8). SdWt was highly correlated with capsule weight (CpWt) and its correlations with TWt and StWt were positive and significant but moderate in magnitude. Therefore, both seed and straw weights can be improved simultaneously and we need only to measure SdWt or CpWt and StWt or TWt, not all four traits.

Due to differences in genetic architecture arising from linkage relationships and possible pleiotropic effects, and because genetic variation was non-significant for some traits, correlations differed a great deal among the crosses (Table 8). However, moderately high correlations of H1, FT, HFT and

Table 7. *Transgression among the F_3 families of the four crosses that showed the best performance for the dual-purpose traits in the 1995 trial*

Cross family	Traits											
	H1*	BR	FT	HFT	H2	CMT	HMT	TWt	NBr	CpWt	StWt	SdWt
						Highest score						
A3 × B3	19·5	3·1	19·9	63·8	55·7	36·2	86·2	270·0	8·5	104·5	193·0	58·5
B3 × L2	16·3	2·7	33·6	83·5	48·3	38·5	96·3	286·5	8·5	128·5	201·0	55·0
A1 × B3	18·5	2·8	30·4	89·0	57·9	41·3	100·9	321·0	10·0	131·5	238·0	68·0
K2 × B3	17·9	2·8	29·3	81·8	51·1	39·2	96·6	299·0	8·5	110·5	200·0	59·5
Best F₃	19·6	3·3	36·0	94·3	80·8	41·3	108·2	389·0	11·0	141·5	256·0	73·5
						Lowest score						
A3 × B3	10·7	1·0	9·5	36·0	34·0	20·0	70·9	40·5	3·0	7·0	7·5	21·0
B3 × L2	10·8	1·2	17·7	64·7	24·6	27·0	80·7	66·5	4·5	20·5	34·0	14·0
A1 × B3	10·6	1·1	11·6	52·4	32·9	27·1	72·7	27·5	4·0	17·0	71·0	25·0
K2 × B3	8·7	1·0	17·3	58·5	26·9	31·1	81·0	145·5	4·5	40·5	82·0	16·0
Worst F₃	7·0	1·0	9·5	35·2	19·7	20·0	53·0	27·5	3·0	7·0	7·5	9·0

* See Table 1 and text for symbols.

Table 8. *Correlations of the seed and straw traits with other characters*

Traits type	Traits										
	H1*	BR	FT	HFT	H2	CMT	HMT	NBr	TWt	CpWt	StWt
CpWt											
Average	**0·67**	**0·68**	**0·56**	**0·63**	**0·60**	**0·71**	0·08	0·28	**0·89**	—	—
Highest	**0·89**	**0·88**	**0·88**	**0·91**	**0·88**	**0·89**	**0·94**	**0·70**	**0·96**		
Lowest	0·25	0·03	−0·36	−0·19	0·28	0·05	0·15	0·05	**0·73**		
StWt											
Average	**0·59**	**0·67**	**0·64**	**0·61**	**0·44**	**0·72**	0·08	0·34	**0·96**	**0·77**	—
Highest	**0·90**	**0·85**	**0·87**	**0·91**	**0·91**	**0·89**	**0·91**	**0·76**	**0·98**	**0·86**	
Lowest	0·02	0·22	−0·17	0·23	−0·26	0·05	0·05	0·00	**0·90**	0·13	
SdWt											
Average	0·20	0·30	−0·07	0·05	0·15	0·15	−0·03	0·05	**0·58**	**0·74**	**0·46**
Highest	0·53	**0·73**	0·29	0·44	0·44	0·38	0·37	0·30	**0·82**	**0·90**	**0·69**
Lowest	0·10	−0·07	−0·45	−0·34	−0·19	−0·43	−0·31	−0·06	0·30	0·49	0·16

* See Table 1 and text for symbols.
Values in bold are significant at $P \leqslant 0.05$, significance of the highest and lowest correlations is adjusted for the fact that they are the most extreme among 11 values.

CMT with CpWt and StWt ($r > 0.59$) suggest that selection indices could be effective in improving all these traits simultaneously. SdWt, on the other hand, cannot be improved through indirect selection because it was not correlated strongly with any of the developmental or maturity traits measured.

Predictions based on the F_3 mean and F_3 genetic variance (see Jinks & Pooni 1980 and Kearsey & Pooni 1996 for procedures) revealed that a higher proportion of the RILs from cross B3 × N2 would meet the dual-purpose criteria compared to those from cross B3 × P2. Approximately 28% of RILs from cross B3 × N2 would score better than the best

parents (see Table 2) for SdWt and StWt simultaneously and the corresponding univariate predictions for the individual traits were 36% and 61% respectively. The proportions of RILs from cross B3 × P2 that would fall under these categories were 10%, 28% and 34% respectively.

The probability of obtaining RILs that would perform better than the best F_1 (0·7% for cross B3 × P2 and 2·8% for cross B3 × N2) was rather low for the dual-purpose traits. Only 2% of the RILs from cross B3 × P2 and 4% from cross B3 × N2 were predicted to have a mean seed weight of 68·0 g/plant or more. However, the proportions predicted for

straw weight were higher (26 % and 53 % respectively) and it would be comparatively easy to produce inbred lines with increased biomass.

Finally, it is clear from the family means that only four F_3 families possessed better dual-purpose characteristics than the best of the F_1s. Two of these families belonged to cross B3 × N2 (families 1 and 19) and the others originated from cross A1 × B3 (families 23 and 25). A bivariate plot also revealed that while many F_3 families performed better than the best F_1 for StWt, only two families (family 11 of cross L2 × P1 and family 1 of cross B3 × N2) had higher seed weights than 68·0 g/plant.

DISCUSSION AND CONCLUSION

The main conclusion from the analysis of early generations (Tables 3 and 4) is that not only are the crosses diverse in their mean performance, they also show segregation, particularly for the seed and straw traits. The existence of heterosis for several traits indicates some genetic diversity among the parental lines and it is possible that inbreeding these crosses would generate new recombinants that combine high seed yield with improved straw/fibre output.

However, all crosses do not show high levels of genetic variation and they differ in their breeding potential (Tables 4 and 5). Some crosses possibly based on closely related parental lines would yield hardly any useful RILs and are not suitable for further breeding such as L2 × P1, A1 × N2 and P1 × P2. On the other hand, crosses B3 × N2 and B3 × P2 possess enough genetic potential for all traits of interest and inbreeding of these crosses should yield RILs of superior quality (see Table 6).

The four most heterotic crosses were disappointing as their F_3 families did not rank high for any trait (see Table 7). This points clearly to the dangers of choosing crosses for further development through inbreeding on the basis of F_1 performance. However, the RILs of these crosses could still produce better dual-purpose varieties compared to existing cultivars and therefore breeding effort would not be wasted.

Finally, the present study has shown clearly that it would be relatively straightforward to combine high seed yield with high straw weight in a single genotype and that genetic correlations would assist in achieving this. Positive correlations between traits should increase the chances of recovering combinations of high seed yield with more straw weight among RILs (see Table 8). Improvements are possible using linseed × linseed or linseed × flax hybrids but not flax × flax crosses because the seed/oil yield of the latter is very low. Inbreeding former crosses should produce RILs which not only possess good dual-purpose characteristics but some may also show superior performance for seed yield and therefore have potential as good linseed varieties.

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