1 Enhancement of genetic diversity in canola-quality *Brassica napus* and *B.*
2 *juncea* by interspecific hybridisation

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15 **Abstract**

16 Reciprocal crosses were made between *Brassica napus* cv. Mystic (canola) and *B.*
17 *juncea* JN29 (near canola quality). The F₁ hybrids were selfed and backcrossed in all
18 possible combinations to parent plants. The greatest number of selfed fertile progeny
19 were obtained when Mystic was the maternal parent, and its F₁ was most successful in
20 backcrosses to Mystic or JN29 as maternal or paternal parent. The predominant
21 morphological type of fertile progeny was *B. napus*, but several *B. juncea*
22 morphological types occurred in F₂ and BC₁ derived lines. F_{2:3} and BC₁S_{0:1} progeny
23 showed transgressive segregation for agronomic and seed quality traits in two
24 contrasting field environments. Several of the *B. juncea*-type progeny had improved

seed quality (lower total seed glucosinolates and higher percent oleic acid) than the *B. juncea* parent. Selfing of interspecific hybrids between canola quality *B. napus* and *B. juncea* has the potential to greatly enhance genetic diversity in canola quality progeny of both species, without the loss of donor alleles that normally occurs with repeated backcrossing.

Additional keywords:

Oilseed rape, Indian mustard, interspecific crossing

Introduction

Oilseed rape or canola (*Brassica napus* L.) is grown in southern cropping regions of Australia with winter dominant rainfall. Traditionally, canola was grown in the higher rainfall cropping areas with up to 700 mm annual average rainfall, but shorter season varieties are now available for regions with 325 mm rainfall (Potter et al. 1999). Canola is a term introduced by the Canola Council of Canada to describe oilseed rape with <2% erucic acid in the seed oil and less than 30 μ moles/g glucosinolates in the meal (Anon. 2007). Canola seed has approximately 60% oleic acid in the oil.

The genetic diversity of Australian breeding populations of *B. napus* has narrowed in the past 30 years during a period of rapid genetic improvement in agronomic traits and conversion to canola quality (Cowling 2007). Several canola varieties released in the late 1990s had a coefficient of ancestry greater than full-sib mating in animals, and the population coefficient of inbreeding after 30 years of closed recurrent selection was 0.21 (Cowling 2007). While this is not a problem with regard to inbreeding defects or lethality, the loss of genetic diversity may limit future

gains in adaptation, yield and disease resistance. A similar diminution of genetic diversity existed in Canadian *B. napus* after decades of seed quality improvement (Downey and Rimmer 1993, Juska et al. 1997). Likewise, three international canola-quality *B. juncea* breeding programs have strong genetic similarities within and between breeding programs, and canola quality *B. juncea* is relatively distant from traditional Indian mustards (Burton et al. 2004).

The *Brassica* secondary gene pool has been a valuable source of genes for improvement of *B. napus*. Traits introgressed into *B. napus* from closely related species include shatter resistance (Prakash and Chopra 1988), earliness (Rao et al. 1993a, 1993b) and blackleg resistance (Chèvre et al. 1997, Roy 1978, 1984). The *Brassica* B-genome has been a useful source of alleles for a number of disease traits in *B. napus* (Plieske et al. 1998). The benefits have flowed in both directions, and recombination with the A genome of *B. napus* may have been responsible for the very low glucosinolates in one *B. juncea*-type progeny of an interspecific cross between a low glucosinolate *B. napus* and a mustard variety of *B. juncea* (Love et al. 1990). However, in most cases, benefits have been derived from introgression of major genes from one species to the other by repeated backcrossing.

Kirk and Oram (1981) identified low erucic acid *B. juncea* lines and combined them with the low glucosinolate lines from Love et al. (1990) to develop low erucic, low glucosinolate *B. juncea* in Australia (Oram et al. 1999). Breeding of canola-quality *B. juncea* continues in Australia with increases in oleic acid to canola standards through crossing with Canadian lines (Burton et al. 2004).

B. juncea would be a valuable source of new genetic diversity in *B. napus*, and vice versa, if extensive backcrossing was not necessary to restore seed quality and agronomic traits. Selfing of interspecific hybrids should promote recombination

among the shared A genomes of the two species, and enhance genetic diversity for complex traits, even if the selfed progeny quickly reverted back to one or the other parental genomes. In a previous study, it was shown that selfed progeny of interspecific hybrids between *B. napus* × *B. juncea* were predominantly *B. napus* genotypes, some with visible introgressions of B genome, and a minority had a full set of B chromosomes and *B. juncea* chromosome number (Schelfhout et al., 2006).

The aim of this work was to demonstrate that genetic diversity for morphological and agronomic traits increased in the selfed and first backcross progeny of *B. napus* × *B. juncea*, while maintaining or improving canola quality. Interspecific progeny were tested as F₂-derived and BC₁S₁-derived progeny in low and high rainfall environments, where transgressive segregants were selected with *B. napus* and *B. juncea* morphology, and tested for seed quality.

Materials and Methods

Crossing regime

F₁ plants from reciprocal crosses between *B. napus* cv. Mystic and *B. juncea* JN29 were selfed and backcrossed to both parents in all possible combinations, with crossing codes and outcomes as described in Schelfhout et al. (2006).

Field trials

F₂ and BC₁ progeny were grown in an insect-proof screenhouse at Shenton Park field station, The University of Western Australia, in 2000 (site code SP2000), and inflorescences were covered with pollination bags to prevent cross-pollination, as described in Schelfhout et al. (2006). Measurements of agronomic traits on F₂ and BC₁ individuals at SP2000 included plant biomass (air dry weight of above ground

dry matter) at harvest, seed yield per plant, number of primary branches, days to anthesis (first open flower), height at harvest and harvest index (seed yield/biomass x 100).

Selfed seed (from inside pollination bags) from fertile F_2 and BC_1 families were advanced in 2001 as $F_{2:3}$ and $BC_1S_{0:1}$ plots in field trials with 2 replicates at 2 sites as described in Schelfhout et al. (2006), with 20 seeds per replicate at Merredin, Western Australia (site code MD2001) and more than 20 seeds at Shenton Park (site code SP2001) where sufficient seed was available. The MD2001 site was located at the Department of Agriculture and Food Western Australia Research Station at Merredin, 250 km east of Perth (31.50 °S, 118.22 °E). This location was a low rainfall cropping site with annual average rainfall 313 mm with 75 per cent falling from April to October (95-year average data from Bureau of Meteorology site number 010093, available on-line at http://www.bom.gov.au/climate/averages/tables/cw_010093.shtml). The SP2001 site was located at The University of Western Australia Field Station, 6 km west of Perth (31.96 °S, 115.79 °E). This site has an annual average rainfall of 705 mm with 90% falling from April to October (39-year average data from Bureau of Meteorology site number 009151, available on-line at http://www.bom.gov.au/climate/averages/tables/cw_009151.shtml). High blackleg disease (causal agent *Leptosphaeria maculans*) pressure was promoted by distributing previous-years infected canola stubble (from *B. napus* cv. Pinnacle) evenly around the entire trial area approximately 4 weeks after seeding. Included in each 2001 trial were 18 selfed individuals from *B. juncea* JN29 and 19 selfed individuals from *B. napus* Mystic from SP2000.

At SP2001 and MD2001, the number of leaves per plant was counted at 4 weeks from sowing, and the average recorded for each plot. At SP2001, blackleg disease survival was calculated by subtracting the number of plants per plot at harvest from the number of plants emerged at 4 weeks, and calculating the percentage survival. At maturity, 6 open-pollinated plants were bulk-harvested from within each plot at each site to calculate the average grain yield and biomass (air dry weight of above ground plant matter) per plant.

Seed quality was analysed on lines with superior agronomic traits and blackleg disease resistance at SP2001 and MD2001. Seed from the bulk harvest of selected plots, or from up to 4 selfed plants per plot, was analysed for oil, protein, glucosinolate and oleic acid content by near infrared radiation (NIR) assay (Foss NIRS 5000) using standard samples for calibration.

Lines were selected at the end of 2001 on the basis of yield, blackleg resistance, seed quality and superior agronomic traits, and promoted to the next generation at Shenton Park in 2002 (SP2002) as $F_{2:4}$ open-pollinated line bulks, $F_{3:4}$ selfed F_3 -derived lines, BC_1S_2 open-pollinated line bulks, or $BC_1S_{1:2}$ selfed BC_1S_1 -derived lines. The SP2002 trial followed the design in SP2001 and included two replicates, with 100 seeds per replicate. Segregation for *B. juncea* and *B. napus* morphology was recorded in the SP2002 field trial. The main purpose of the SP2002 trial was to harvest superior agronomic lines and analyse harvested seed for oil, protein, and glucosinolates by NIR and fatty acid profile by gas chromatography (GC) on a GC-17A V3 (Shimadzu) equipped with flame-ionization detector and flow splitter. Results for fatty acid content from GC were integrated by GC solution software (Shimadzu).

Analysis of field trial data

Column scatter plots were used to illustrate the mean and range of variation in the parent populations and F_2 and BC_1 families in SP2000. Fisher's protected least significant difference test was used to demonstrate similarities or differences between family means for each agronomic trait.

Principal components analysis (PCA) was used to summarize associations among progeny and parents based on the mean value across replicates of agronomic traits measured on plots at SP2001 and MD2001. Data were standardized for mean and variance before analysis by Genstat software (VSN International, UK).

Results

Morphological attributes of parental lines

B. napus cv. Mystic was characterised by medium plant stature, dark green ovoid shape leaves with smooth serrations, and long non-constricted siliqua with black seeds. *B. juncea* JN29 plants were 26% taller on average than Mystic (Fig. 1), with light green elongated leaves and pronounced serrations. Siliqua of JN29 were more constricted around the seed than Mystic, with mustard yellow seeds, and were resistant to shattering at maturity. The flowering time of JN29 was similar to Mystic however the harvest index was considerably lower (Fig. 1).

Agronomic traits of F_2 and BC_1 progeny

F_1 plants of cross 99X022 (Mystic/JN29) were more fertile on selfing and backcrossing than F_1 plants of the reciprocal cross 99X055 (JN29/Mystic) (Schelfhout et al. 2006). One backcross family (00X014) failed to germinate in July 2000 (Schelfhout et al. 2006), and did not contribute further to results.

The dominant morphological type of the F₂ and BC₁ progeny in SP2000 was that of the *B. napus* parent Mystic. However, in later generations, some *B. juncea* morphological types were observed in a minority of selfed (99X022 and 99X055) and backcross progeny. Backcross family 00X013 (Mystic/JN29//JN29) showed only *B. juncea*-type morphology. Lines from 00X013 had high scores for height and low scores for harvest index, similar to the *B. juncea* parent JN29. However, these lines were low in fertility and did not survive to future generations (Schelfhout et al. 2006). Lines segregating for *B. juncea* morphology at SP2002 were:

00X011-022, -076, -127, -140	(BC ₁ -derived ex Mystic/JN29//Mystic)
00X012-046, -141, -152	(BC ₁ -derived ex Mystic//Mystic/JN29)
99X022-10, -44, -53, -58, -93	(F ₂ -derived ex Mystic/Jn29)
99X055-04	(F ₂ -derived ex Jn29/Mystic)

<<Fig. 1 here...>>

F₂ and BC₁ families at SP2000 varied significantly in dry biomass at maturity, harvest index, height at maturity, and days to flowering (Fig. 1). Plant seed yield differences were also observed among families (Schelfhout et al. 2006). The selfed family with the highest mean biomass was 99X022 (Mystic/JN29) and its backcross progeny, especially 00X012 (Mystic/JN29//Mystic), which had the highest mean biomass (greater than the parents and self or backcross families) and showed transgressive segregation above the parents (Fig. 1). F₂ family 99X055 (JN29/Mystic) and its backcrosses tended to be low yielding or to produce unviable seed (00X017), except for 00X015 (JN29/Mystic//JN29) which had similar mean yield and biomass per plant to the parents, but only had 6 surviving plants (Fig. 1).

B. napus cv. Mystic plants had greater harvest index on average (14.6%) than *B. juncea* JN29 (11.6%). Mean harvest index was between 10 and 15 percent for most F_2 and BC_1 families except those with poor fertility.

B. juncea JN29 plants were 40 cm taller on average than *B. napus* cv. Mystic plants in SP2000, with large variation in height in both parental lines (Fig. 1). Most F_2 and BC_1 families had a mean height between the two parents, except for 00X013 (Mystic/JN29//JN29) which was 40 cm taller on average than the *B. juncea* JN29 and 80 cm taller on average than *B. napus* cv. Mystic. Flowering date was significantly later in all F_2 and BC_1 families than in the parents, and the range of flowering dates among *B. juncea* JN29 plants (67 – 101 days) was much wider than among *B. napus* cv. Mystic plants (69 – 86 days) (Fig. 1).

Agronomic traits in $F_{2:3}$ and $BC_1S_{0:1}$ field trials

Strong positive correlations between yield per plant and biomass per plant in SP2001 and MD2001 are reflected by the close association of vectors for these traits in PCA plots for SP2001 (Fig. 2) and MD2001 (Fig. 3). The vectors for yield and harvest index at both sites were less strongly associated, and there was a weak negative association between yield and flowering date at SP2001. Yield was independent of height at both sites, and independent of blackleg disease resistance at SP2001 (as indicated by perpendicular vectors).

The ellipses on the PCA graphs in Fig. 2 (SP2001) and Fig. 3 (MD2001) indicate the range of positions for most of the selfed plants of *B. juncea* JN29 and *B. napus* cv. Mystic, and there was no overlap between the two parents. Two control varieties of *B. napus*, cv. Surpass 400 and cv. Monty, fell within the range of values recorded for *B. napus* cv. Mystic (Figs. 2 and 3). JN29 plants were associated with

the height vector (taller than Mystic plants), had more leaves per plant at 4 weeks, and tended to have more biomass than Mystic plants, but both species had a similar range in yield at both locations. *B. juncea* JN29 plants had lower harvest index than *B. napus* cv. Mystic plants, were later flowering, and had stronger blackleg resistance at SP2001 (Fig. 2).

$F_{2:3}$ and $BC_1S_{0:1}$ progeny tended to be distributed between the two parent ranges, with some inside each range, at SP2001 (Fig. 2) but most tended to cluster within the *B. napus* parent range at MD2001 (Fig. 3) where blackleg resistance and flowering date were not measured (very little blackleg was observed at MD2001). $F_{2:3}$ progeny from cross 99X022 at SP2001 were associated with the vectors for longer days to flowering and lower yield (Fig. 2). Backcross progeny appeared to be more strongly associated with yield and harvest index (towards the *B. napus* range) in both SP2001 and MD2001 trials.

The best yielding lines in 2001 were predominantly $BC_1S_{0:1}$ progeny with few $F_{2:3}$ progeny in these regions of the PCA graphs. Some $BC_1S_{0:1}$ and $F_{2:3}$ progeny were higher yielding than the parental lines, especially in the drought-stressed environment of MD2001.

<<Fig. 2 goes here.....>>

<<Fig. 3 goes here.....>>

Seed quality analysis

Seed harvested from parent selections, $F_{2:3}$ and $BC_1S_{0:1}$ lines at SP2001 consistently produced higher seed oil, higher oleic acid, lower seed protein and lower glucosinolates in seed than those in the MD2001 trial (Table 1). There was a strong

inverse relationship between protein and oil content at the two sites, but the combined seed {oil + protein} was consistent between sites, with *B. napus* cv. Mystic selections achieving the same value of 70.5% {oil + protein} at both sites (Table 1).

B. napus cv. Mystic lines were 3-4% higher in seed oil, 2-3% lower in seed protein, 1% higher in seed {oil + protein}, 12% higher in oleic acid, and significantly lower in glucosinolate content than *B. juncea* JN29 at the two trial locations (Table 1). At MD2001, some individuals in families 00X011 and 00X012 had higher oil, higher protein and higher oleic acid contents than Mystic, with glucosinolate content equal to or lower than Mystic, and at both MD2001 and SP2001 some individuals exceeded the seed {oil + protein} percentage of Mystic. Lines harvested from the SP2001 trial did not show the same extremes in quality values as those harvested from MD2001.

<<< Table 1 goes here >>>>

Of the 6 highest yielding interspecific progeny in MD2001, all were *B. napus* morphotypes, 5 were BC₁-derived and 1 was F₂-derived (Table 2). These lines out-yielded the parent lines, and had equal or higher oil, oleic acid and protein contents than Mystic and lower glucosinolate content than JN29 (Table 2).

Two lines harvested from the BC₁S₂ and F₄ generation at SP2002 (99X022-093-3 and 99X055-004-5) were high-yielding *B. napus* morphotypes that had high glucosinolates similar to the *B. juncea* parent (Table 2). Some *B. juncea* morphotypes in SP2002 (00X011-022-4, 00X012-141-3, and 99X022-058M2-3) had canola quality seed composition with low glucosinolate levels (Table 2). All lines in Table 2 had zero detectable erucic acid content.

Genetic segregation for seed quality traits within families was also evident. $F_{2:3}$ line 99X022-044 (*B. juncea* morphotype) had low oil, high protein and high glucosinolates in seed harvested from MD2001, whereas selfed single plant selections from MD2001 (99X022-044-1 and 99X022-044-3) were segregating for oil, protein and glucosinolates in seed harvested from SP2002, but retained their *B. juncea* morphotype (Table 2).

<<< Table 2 goes here >>>

Discussion

Enhanced genetic variation for key agronomic and seed quality traits was present in selfed progeny of interspecific hybrids between *B. napus* (canola) $\times$ *B. juncea* (near-canola quality). *B. napus* was the predominant morphotype in selfed and backcrossed progeny, and most of these lines retained their canola quality. Genetic variation was present also among rare *B. juncea* morphotypes among the selfed interspecific progeny, and the seed quality of some *B. juncea*-type progeny was improved beyond the *B. juncea* parent with lower glucosinolates and higher oleic acid. All progeny had zero detectable erucic acid. Transgressive segregation for agronomic and quality traits was evident among the selfed and backcross interspecific progeny.

The most successful primary cross direction was with *B. napus* as the maternal parent, and the most successful backcross parent was *B. napus*. This contrasts with results of the similar interspecific crosses by Roy (1980), and agrees with the conclusion of Patil et al. (2003), that the most successful primary cross direction is genotype dependent.

Interspecific hybridisation has been used to introduce new genes for shatter resistance, earliness and blackleg disease resistance into *B. napus* from *B. juncea* or *B. carinata* and other *Brassica* species (Prakash and Chopra 1988, Rao et al. 1993a, 1993b, Chèvre et al. 1997, Roy 1978, 1984). Resynthesis of the amphidiploid *B. napus* from the two diploid progenitor species (*B. rapa*, *B. oleracea*) also introduced new genetic variation into *B. napus* (Chen and Heenan 1989). The usefulness of interspecific crossing in *Brassica* is often limited by low fertility in progeny, and embryo rescue or other interventions are often required (Kumar et al. 1988). However, the major problem for canola breeders is the lack of canola quality progeny in such wide crosses. Fertile, canola-quality and genetically stable *B. napus*-type progeny are rare, and breeders generally undertake extensive backcrossing to the *B. napus* parent in order to restore canola quality.

In these experiments, genetic variation was introduced into selfed *B. napus*-type canola quality progeny following wide crossing with *B. juncea* – when the *B. juncea* parent was near canola-quality. This new genetic diversity from *B. juncea* was immediately available for exploitation in *B. napus* breeding. This increases the potential to retain potentially useful alleles from the donor parent, compared with continued backcrossing to the *B. napus* parent. The majority of selfed interspecific progeny were *B. napus* morphology, fertile with high seed yield, biomass and harvest index (Fig. 1). A minority of F₂-derived or BC₁-derived progeny had *B. juncea* morphology, and some of these had better seed quality (higher seed oil, higher oleic acid and lower glucosinolates) than the *B. juncea* parent. Benefits from interspecific crossing with canola quality parents of *B. juncea* and *B. napus* will be possible in both species.

Some selfed progeny had *B. juncea* morphology (leaf shape and colour, seed colour, plant morphology), a chromosome count of $2n = 38$ and evidence for B-genome introgression, but lacked complete B-genome chromosomes (Schelfhout et al. 2006). These results indicate that *B. juncea* morphology does not depend on the presence of a complete set of B genome chromosomes.

Our data on seed quality in selections from interspecific crossing were obtained from two locations in 2001, and further years and locations would be necessary to obtain definitive information on seed quality of particular selections. However, there were many consistencies in the ranking of lines for quality across the two sites, and some general conclusions can be drawn. The seed quality of *B. juncea* parent JN29 was close to canola (Anon. 2007) but with elevated glucosinolates and lower oleic acid% in oil (Tables 1 and 2). The level of glucosinolates in JN29 is above the recommended level for use of the term “canola” (Anon. 2007). Interspecific crossing with canola (*B. napus*) cv. Mystic resulted in *B. juncea*-type progeny with lower levels of seed glucosinolates and higher levels of oleic acid than JN29 (Table 2). *B. napus* was a useful source of seed quality alleles for *B. juncea* in this interspecific crossing program.

There was evidence for transgressive segregation for important agronomic and seed quality traits in both species following this cross. Evidence was presented for a B-genome repeat sequence (Schelfhout et al. 2004) or partial introgressions in *B. napus*-type selfed progeny (Schelfhout et al. 2006). Another source of variation is recombination between the A-genome chromosomes of *B. juncea* and *B. napus* during meiosis in the F_1 interspecific hybrid. F_3 -derived progeny 99X055-004-5, which was derived from *B. napus* cv. Mystic as pollen parent and *B. juncea* JN29 as maternal parent, had *B. napus* morphology and *B. juncea* seed composition (Table 2).

Breeders should avoid the temptation to eliminate lines following interspecific crossing based on visual observations or seed quality assessment in early generations. Potentially valuable lines may have their potential masked by epistatic or dominance effects, and continued selfing and selection is necessary to reveal the full potential of these crosses. For example, progeny 99X022-044 in the F₃ generation at MD2001 had elevated glucosinolates and low seed oil, but segregation occurred between two F_{3:4} progeny at SP2002 for glucosinolates and both had higher seed oil than the parent line (Table 2).

Principal component analysis was a useful tool for displaying trends in agronomic traits in interspecific progeny versus parents, and for the selection of elite lines in target environments. It also showed the range of attributes among selfed lines from *B. juncea* JN29 and *B. napus* cv. Mystic. *B. juncea* is considered a potentially useful species for low rainfall and higher temperature environments (Woods et al. 1991), and therefore work is underway to improve this species for Australian cropping systems (Burton et al. 1999, 2004). Traditionally *B. juncea* is grown in India under dry and heat stress conditions, and we anticipated that *B. juncea* would be a source of useful alleles for low rainfall environments in Australia, which suffer from moisture and heat stress. This was shown to be true in *B. napus*-type interspecific progeny at MD2001, where some progeny showed greater tolerance of drought stress than the *B. napus* parent Mystic.

These results demonstrate that it is possible to make agronomic gains from interspecific crossing without compromising the quality of oilseed *Brassica*. The yellow seeded *B. juncea* used in this study is likely to be derived from a similar lineage to the ZEM 1 and ZEM 2 lines reported by Kirk and Oram (1981). The tall stature and yellow seeds of JN29 are characteristic of the China / Eastern Europe

group identified by Vaughan (1963). *B. juncea* JN29 is inefficient at converting biomass to yield. This is reflected in the low harvest index values, and tall growth. Future genetic selections of canola quality *B. juncea* with shorter stature and drought and heat tolerance may provide more useful alleles for *B. napus* canola breeding than described in this paper.

B. juncea accessions from the India / Pakistan group (Vaughan, 1963) are better adapted to shorter seasons and higher temperatures than their more northern relatives, but are not canola quality. Access to these alleles in *B. napus* may be possible via interspecific crossing with canola quality *B. juncea*, but this would require more diverse germplasm in canola quality *B. juncea* than currently exists. This flow of alleles across species boundaries will be accelerated by selfing of interspecific hybrids and reselection of canola quality progeny.

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496 Table 1

Family ^A	Site MD2001			Site SP2001		
	No. lines	Mean	Range	No. lines	Mean	Range
Seed oil (% dry weight)						
Mystic	4	45.1	43.6 – 45.8	4	53.9	52.3 – 55.2
JN29	4	40.4	37.5 – 43.3	4	50.3	48.7 – 50.9
99X022	11	41.7	38.1 – 45.0	10	51.6	46.9 – 54.7
99X055	2	38.8	36.5 – 41.1	1	51.5	
00X015	3	43.5	42.4 – 45.1	3	53.2	49.9 – 55.3
00X013	1	45.5		1	53.0	
00X012	44	42.9	38.8 – 47.5	46	50.9	42.8 – 55.4
00X011	28	43.9	41.3 – 47.1	29	52.0	46.9 – 55.9
Seed protein (% dry weight)						
Mystic	4	25.4	24.8 – 26.5	4	16.6	16.0 – 17.8
JN29	4	29.3	26.7 – 31.6	4	18.3	17.6 – 19.3
99X022	11	27.8	25.3 – 28.9	10	18.1	15.7 – 20.1
99X055	2	29.5	28.6 – 30.4	1	18.2	
00X015	3	27.1	26.2 – 28.1	3	17.2	15.8 – 19.2
00X013	1	25.6		1	17.3	
00X012	44	27.2	24.7 – 29.2	46	18.7	15.2 – 24.6
00X011	28	26.7	24.5 – 29.4	29	17.9	15.2 – 21.8
Seed oil + protein (% dry weight)						
Mystic	4	70.5	70.1 – 70.8	4	70.5	69.9 – 71.2
JN29	4	69.7	69.1 – 70.0	4	68.6	68.0 – 69.6
99X022	11	69.5	66.4 – 72.9	10	69.7	67.0 – 71.2
99X055	2	68.3	66.9 – 69.8	1	69.7	
00X015	3	70.6	70.3 – 70.5	3	70.4	69.0 – 71.1
00X013	1	71.1		1	70.3	
00X012	44	70.1	66.2 – 73.8	46	69.6	66.3 – 73.0
00X011	28	70.6	68.1 – 72.6	29	69.9	68.5 – 71.4
Seed glucosinolates (μmol/g)						
Mystic	4	8.1	3.2 – 11.3	4	2.5	0.7 – 3.4
JN29	4	44.5	34.3 – 57.6	4	43.8	37.8 – 48.8
99X022	11	11.7	4.7 – 37.6	10	3.9	1.8 – 7.2
99X055	2	8.2	6.6 – 9.7	1	4.2	
00X015	3	6.1	4.5 – 7.0	3	2.3	0.6 – 3.5
00X013	1	2.3		1	2.2	
00X012	44	7.8	1.1 – 13.9	46	4.0	1.4 – 7.0
00X011	28	7.4	2.3 – 12.1	29	5.1	1.3 – 8.8
Oleic acid (%fatty acid composition)						
Mystic	4	59.2	56.7 – 61.4	4	61.1	60.1 – 61.8
JN29	4	46.3	42.2 – 49.1	4	49.4	48.7 – 50.9
99X022	11	53.8	47.7 – 59.6	10	58.7	51.0 – 62.5
99X055	2	50.9	46.0 – 55.7	1	60.6	
00X015	3	57.6	54.8 – 62.4	3	59.7	52.2 – 63.6
00X013	1	57.6		1	65.1	
00X012	44	57.8	50.8 – 65.8	46	59.0	48.7 – 65.7
00X011	28	61.1	54.4 – 66.7	29	62.6	56.2 – 66.8

497 ^AThe full cross pedigrees for families are provided in Schelfhout et al. (2006)

Table 2

Selections within families ^A	Generation	Site and year	Oil (%)	Protein (%)	Oil + protein (%)	Oleic acid (%)	Glucosinolates (μmol/g)	Morphology ^B
High yielding accessions at MD2001:								
00X011-127	BC ₁ S ₁	MD2001	42.8	29.4	72.2	61.7	6.6	<i>B. napus</i>
00X011-084	BC ₁ S ₁	MD2001	47.1	24.8	71.9	66.7	1.7	<i>B. napus</i>
00X011-116	BC ₁ S ₁	MD2001	46.5	25.4	71.9	62.7	2.3	<i>B. napus</i>
00X012-037	BC ₁ S ₁	MD2001	46.8	25.4	72.2	63.5	6.3	<i>B. napus</i>
00X012-105	BC ₁ S ₁	MD2001	45.5	25.5	71.0	60.4	7.0	<i>B. napus</i>
99X022-147	F ₃	MD2001	44.6	28.3	72.9	59.6	4.9	<i>B. napus</i>
Accessions with unusual traits at SP2001, SP2002 and MD2001:								
99X022-093-3	F ₄	SP2002	40.7	25.9	66.6	58.1	47.0	<i>B. napus</i>
99X055-004-5	F ₄	SP2002	40.4	25.4	65.8	50.8	42.0	<i>B. napus</i>
00X011-022-4	BC ₁ S ₂	SP2002	48.6	19.0	67.6	46.4	2.0	<i>B. juncea</i>
00X012-141-3	BC ₁ S ₂	SP2002	48.8	18.9	67.7	63.0	2.0	<i>B. juncea</i>
99X022-058M2-3	F ₄	SP2002	49.8	18.6	68.4	46.1	8.0	<i>B. juncea</i>
99X022-044	F ₃	MD2001	43.5	27.7	71.2	53.2	37.6	<i>B. juncea</i>
99X022-044-1	F ₄	SP2002	46.0	22.2	68.2	43.7	40.0	<i>B. juncea</i>
99X022-044-3	F ₄	SP2002	49.2	18.5	67.7	47.0	18.0	<i>B. juncea</i>

^AThe full cross pedigrees for families are provided in Schelfhout et al. (2006)

^BMorphology relates to the parent-type. For *B. juncea*, this includes *B. juncea* parental leaf shape and colour, plant morphology, and seed colour (yellow). For *B. napus*, the parental leaf shape and plant morphology were accompanied by *B. napus* seed colour (black or dark brown).

Figure and Table Captions

Figure 1. Column scatter plots of agronomic measurements on F₂ or BC₁ plants from the interspecific hybridisation of *B. napus* cv. Mystic × *B. juncea* JN29 at SP2000.

Agronomic traits included number of days to anthesis, mature plant biomass (g/plant), harvest index (%), and height at maturity (cm). The mean of each family is indicated by the horizontal bar in the vertical column of data points from each family, and letters adjacent to the mean indicate differences among the means according to Fisher's protected least significant difference test ($P = 0.05$). Codes for cross families are abbreviated from Schelfhout et al. (2006), where "11" = 00X011, "12" = 00X012, "13" = 00X013, "15" = 00X015, "16" = 00X016, "17" = 00X017, "18" = 00X018, "22" = 99X022, "55" = 99X055, and "Mys" = Mystic.

Figure 2. Principal components analysis biplot of line means for agronomic and disease traits measured on interspecific hybrid progeny at SP2001. Progeny data points are plotted on the primary x-y axes and trait vectors are plotted on the secondary x-y axes. Abbreviations: "Yld" = yield (g) per plant, "HI" = harvest index, "DtF" = days to first flowering, "BL" = blackleg resistance rating, "Ht" = height at maturity, "Lvs" = leaf number 4 weeks after sowing, "BM" = whole plant biomass at harvest (g), "S400" = *B. napus* cv. Surpass 400.

Figure 3. Principal components analysis biplot of line means for agronomic and disease traits measured on interspecific hybrid progeny at MD2001. Progeny data points are plotted on the primary x-y axes and trait vectors are plotted on the secondary x-y axes. Abbreviations: "Yld" = yield (g) per plant, "HI" = harvest index,

“Ht” = height at maturity, “Lvs” = leaf number 4 weeks after sowing, “BM” = whole plant biomass at harvest (g), “S400” = *B. napus* cv. Surpass 400.

Table 1. Mean and range of seed composition (NIR assay) of families from the interspecific cross *B. napus* cv. Mystic $\times$ *B. juncea* JN29 at SP2001 and MD2001. Seed composition was assessed on harvested samples from the most promising progeny at each site, based on agronomic observations before harvest.

Table 2. Seed composition (NIR assay) of notable individual interspecific progeny of *B. napus* cv. Mystic $\times$ *B. juncea* JN29 from SP2001, MD2001 and SP2002. Values for parent lines are as listed in Table 1.

Fig. 1

Fig. 2

Fig. 3