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Transgressive segregation of erucic acid content in *Brassica carinata* A. Braun

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Abstract Two Ethiopian mustard (*Brassica carinata* A. Braun) lines with low (about 10%) and zero erucic acid (C22:1) have been obtained. The low C22:1 mutant line L-2890 was isolated after a chemical-mutagen treatment of C-101 seeds (about 40% C22:1). The zero C22:1 line L-25X-1 was obtained by interspecific crossing. Our objective was to determine the genetic control of low and zero C22:1 contents in these lines and the relationship between the loci controlling these traits. Reciprocal crosses between L-2890, L-25X-1 and high C22:1 lines, and between L-2890 and L-25X-1, were made. The F_1, F_2 and BC_1 F_1 generations were obtained. No maternal or cytoplasmic effects for C22:1 content were observed in any of the crosses. The analysis of the fatty acid composition in the segregating populations from the crosses of L-2890 with the high C22:1 lines C-101 and L-1630 indicated that the segregation patterns fitted a model of two alleles at two loci, *M1* and *M2*, with partial (near complete) dominance for high concentration. The segregation patterns in the cross of the zero C22:1 line L-25X-1 with the high C22:1 line L-1630, were explained on the basis of two genes, *E1* and *E2*, with additive gene action. The F_1 and segregating generations of the crosses L-2890 × L-25X-1 showed a strong transgressive segregation with C22:1 values of up to 50.0%, four-fold higher than those of L-2890. The analyses of the F_2, BC_1F_1 and F_3 generations indicated that the combination of alleles at four loci, M_1 and M_2 in L-2890 and E_1 and E_2 in L-25X-1, controlled the transgressive segregation for C22:1. The proposed genotypes (C22:1 content) for each parent were as follows: L-2890 (10% C22:1) = $m_1m_1m_2m_2E_1E_1E_2E_2$; L-25X-1 (0% C22:1) = $M_1M_1M_2M_2e_1e_1e_2e_2$; and C-101 (45% C22:1) = $M_1M_1M_2M_2E_1E_1E_2E_2$.

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Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) is a minor oilseed species indigenous to Ethiopia with a high potential as an oil crop for the rain-fed Mediterranean area. Under semi-arid conditions, it has several desirable agronomic characteristics compared to other *Brassica* crops: the root system is more highly developed and aggressive than in *Brassica napus*, the plant is resistant to drought, pod shattering and to a wide range of diseases and pests, and it has a higher yield potential (Fereres et al. 1983; Malik 1990; Getinet et al. 1997). The Ethiopian mustard cultivars grown in Ethiopia have an oil content of up to 42% (Westphal and Marquard 1980). Despite its agronomic interest, major limiting factors for a wider usage of this species have been the naturally high levels of erucic acid and glucosinolates in its seed. The erucic acid (C22:1) content of the seed oil of traditional genotypes of *B. carinata* ranges from 35 to 45% of the total fatty acid composition (Mnzava and Olsson 1990; De Haro et al. 1998; Becker et al. 1999), which is undesirable in a vegetable oil for human consumption (Vles 1974).

Therefore, efforts have been made to develop low erucic-acid genotypes of *B. carinata* using different strategies. Interspecific crossing with *B. napus* and *Brassica juncea* (Fernández-Escobar et al. 1988; Fernández-Martínez et al. 2001) or with *B. juncea* (Getinet et al. 1994), or cross-breeding and continuous pedigree selection within the *B. carinata* germplasm (Alonso et al. 1991), have permitted the development of zero C22:1 *B. carinata* lines. Mutagenesis has also facilitated the development of *B. carinata* mutant lines with low (<10%) C22:1 from material with standard levels (Velasco et al. 1995)

One requisite for the incorporation of low C22:1 content into commercial cultivars is a previous knowledge of the genetic behaviour of the trait. Previous studies on the genetics of C22:1 in the amphidiploids *B. napus* (Harvey and Downey 1964) and *B. juncea* (Kirk and Hurlstone 1983) have shown that, in both species, it is controlled by alleles at two loci acting in an additive manner, while in the diploid *Brassica rapa* species it is controlled by a single additive gene (Dorrell and Downey 1964). No maternal or cytoplasmic effects were observed in these studies. Limited genetic studies have been conducted to-date on the inheritance of the C22:1 content in the seed oil of Ethiopian mustard. Getinet et al. (1997) investigated the inheritance of erucic acid content in the progeny of crosses between the high-C22:1 *B. carinata* cultivars Dodolla and S-67 with the zero-C22:1 line C90-14. They found that, as in *B. napus* and *B. juncea*, the C22:1 concentration in *B. carinata* was controlled by two genes acting in an additive manner with each allele contributing about 10% erucic acid. Alemayehu and Becker (2001) also reported digenic control and additive gene action for C22:1 content in crosses involving low and high C22:1 *B. carinata* lines, and suggested the presence of alleles with a different individual contribution in these lines.

An Ethiopian mustard mutant line L-2890 with low C22:1 levels (of about 10%) was obtained following EMS treatment of line C-101 seeds (about 40% of C22:1) (Velasco et al. 1995). The zero C22:1 line L-25X-1 was developed from interspecific crosses of high C22:1 *B. carinata* and zero C22:1 *B. napus* and *B. juncea* (Fernández-Martínez et al. 2001). Since L-2890 was developed independently from L-25X-1, it was assumed that both lines would have different genetic systems controlling C22:1. The objectives of this research were: (1) to study the genetic control of low and zero C22:1 in L-2890 and L-25X-1; and (2) to study the relationship between the genetic systems controlling the C22:1 in these lines.

Materials and methods

Plant material

The lines used in this study were the low C22:1 mutant line L-2890 obtained after mutagenic treatment with EMS (Velasco et al. 1995), its parental line C-101 (standard C22:1 content), the zero C22:1 line, L-25X-1, obtained from interspecific crosses of *B. carinata* line C-101 and zero C22:1 *B. juncea* and *B. napus* (Fernández-Martínez et al. 2001), and the very high C22:1 line L-1630 also obtained by mutagenesis (Velasco et al. 1998). The fatty acid composition of these lines is shown in Table 1.

Genetic study

Reciprocal crosses were made between: (1) line L-2890 and the high C22:1 lines C-101 and L-1630 in order to study the inheritance of low, high and very high C22:1 contents, (2) between L-25X-1 and L-1630 to study the genetic control of zero and very high C22:1 content, and (3) between L-25X-1 and L-2890 to clarify the relationship between the low and zero C22:1 traits. The seeds of L-2890, L-25X-1, C-101 and L-1630 were individually analysed for fatty acid composition by the half-seed technique (Thies 1971) to ensure that the plants used for the inheritance study were breeding true for their low, zero and high C22:1 contents, respectively.

Half-seeds of each parental line were germinated, grown in a growth chamber and, at the stage of four true leaves, transplanted into pots in the winter of 1994. The plants were grown under greenhouse conditions at 22/18 °C (day/night) with an 18-h day length. Crossing was achieved through the emasculation of immature flower buds of the female parent followed by immediate pollination of their stigmas with fresh pollen from the male parent. Selfing was achieved by pollinating immature flower buds with pollen from open flowers of the same plant. Crosses, as well as selfed buds, were covered with paper bags to prevent any contamination from external pollen. The fatty acid composition of F_1 half-seeds from each cross was analysed by gas-liquid chromatography (GLC). The parents and F_1 half-seed from each reciprocal cross, were grown in the greenhouse in winter 1995 (crosses L-2890 × L-25X-1, L-2890 × L-1630 and L-25X-1 × L-1630), and in winter 1996 the cross L-2890 × C-101. F_1 plants were self–pollinated to produce F_2 seed and reciprocally backcrossed to both parents to obtain BC_1F_1 seed. Reciprocal crosses between the two parents were repeated to obtain F_1 seeds on the same environment as F_2 and BC_1F_1 seeds. An evaluation of the fatty acid composition at the F_1 plant level was made by averaging the GLC analyses of the F_2 seeds from each individual F_1 plant. Fatty acid composition was determined from a total of 311 individual F_2 seeds, and a total of 271 BC_1F_1 seeds from the backcrosses to both parents of the cross L-2890 × C-101, 580 F_2 seeds and 855 BC_1F_1 seeds of cross L-2890 × L-1630, 748 F_2 seeds and 1,017 BC_1F_1 seeds of the cross L-25X-1 × L-1630, and 885 F_2 seeds and 927 BC_1F_1 seeds of the cross L-2890 × L-25X-1.

Table 1 Mean ± standard deviation of fatty acid composition of seed oil of (*B. carinata*) Ethiopian mustard lines C-101, L-2890, L-25X-1 and L-1630 grown in the greenhouse

Lines[a]	N[b]	n[c]	Fatty acids (% of total)[d]						
			C22:1	C20:1	C18:1	C18:2	C18:3	C16:0	C18:0
L-25X-1	3	50	0.04 ± 0.1	1.1 ± 0.3	24.9 ± 3.0	45.9 ± 4.3	19.8 ± 2.8	6.1 ± 0.9	1.3 ± 0.3
L-2890	3	50	9.7± 1.3	9.9 ± 1.0	17.3 ± 2.4	28.2 ± 2.0	24.6 ± 2.5	5.8 ± 0.6	1.1 ± 0.1
C-101	3	25	45.1± 2.5	5.3 ± 0.9	9.4 ± 0.9	23.2 ± 3.7	11.1 ± 2.3	2.8 ± 0.7	0.5 ± 0.1
L-1630	3	75	54.8 ± 2.3	5.6 ± 0.9	11.0 ± 1.6	7.8 ± 1.6	16.5 ± 2.1	2.5 ± 0.2	0.3 ± 0.1

[a] C-101: parental line; L-25X-1: derived from interspecific crosses between high erucic acid *B. carinata* and zero erucic acid *B. napus* and *B. juncea*; L-2890 and L-1630: mutant lines derived from C-101
[b] Number of single plants analysed
[c] Number of half-seeds analysed within each plant
[d] Does not include minor fatty acids: myristic, arachidic, palmitoleic, cis-11, 14-eicosadienoic and nervonic acids

A total of nine F_2 half-seeds of the cross L-2890 × L-25X-1, representing all the classes for C22:1 concentration detected in this generation, were selected, germinated and grown in the greenhouse in winter 1997 to obtain the F_3 generation. The study of this generation was performed through the analysis of about 60–90 F_3 seeds from each segregating F_2 plant and about 25–40 seeds from each non-segregating F_2 plant.

Statistical analyses

Means of the C22:1 content were calculated in the parental and F_1 generations, and compared by using the *t*-test. Since the results did not reveal any maternal effects for the C22:1 content the fatty acid composition of segregating generations was analysed on single seeds. The C22:1 content of BC_1F_1, F_2 and F_3 seeds was assigned to phenotypic classes on the basis of the appearance of discontinuities in the frequency distribution and the values found in the parentals grown under the same environmental conditions. The proportion of seeds observed in each phenotype class was compared to those expected on the basis of appropriate genetic hypotheses. The goodness-of-fit to tested ratios was measured by the chi-square statistic. Heterogeneity χ^2 for families within a cross was non-significant so that data for families for the same cross were pooled for analysis.

Fatty acid analyses

Fatty acid methyl esters were obtained as described by Garces and Mancha (1993) and analysed on a Perkin Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk,USA) equipped with a flame ionization detector (FID) and a 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Incorporated, Bellefonte, USA). The oven, injector and flame ionization detector were held at 195 °C, 275 °C and 250 °C, respectively.

Results and discussion

Genetic control of the low (about 10%) C22:1 content in EMS mutant line L-2890 in crosses with high erucic acid parents C-101 (45%) and L-1630 (55%)

Table 1 shows the fatty acid composition of the seed oil of the low C22:1 content mutant L-2890, its parental line C-101 and the very high C22:1 line L-1630, all grown in the same environment. The three lines differed in the proportion of most of the other fatty acids. Table 2 shows the C22:1 contents of the seed oil of the parents and of the F_1 seed and F_1 plants (F_2 seeds averaged) from their reciprocal cross in low × high, and low × very high crosses. The variation in the C22:1 content between different L-2890 plants observed in Table 2 was attributed to environmental effects. No reciprocal differences for C22:1 levels in the F_1 seeds or in the F_1 plants were observed for any of the crosses, indicating the absence of maternal and cytoplasmic effects for erucic acid content. These results are in agreement with findings previously reported in *B. carinata* (Getinet et al. 1997), *B. napus* (Harvey and Downey 1964; Kondra and Stefansson 1965), *B. rapa* (Dorrell and Downey 1964) and *B. juncea* (Kirk and Hurlstone 1983). Since no significant maternal or cytoplasmic effects could be detected on the C22:1 content in any of the four crosses, the data from reciprocal F_1, F_2 and BC_1F_1 seeds were combined in Fig. 1A, B.

F_1 seeds from crosses between L-2890 × C-101 and L-2890 × L-1630 showed the C22:1 mean value, which were intermediate between the parents but much higher than the midparent value (Table 2 and Fig. 1A, B) indicating that the high C22:1 content in C-101 and L-2890 is partially (near completely) dominant over the low C22:1 content in L-2890. The analysis of individual F_2 seeds of the L-2890 × C-101 and L-2890 × L-1630 crosses showed a clear bimodal pattern for C22:1 content (Fig. 1A, B). The first class was assigned to the "low" category (C22:1 < 13%), corresponding to the range

Table 2 Mean ± standard deviation of erucic acid content of the parents L-2890, C-101, L-25X-1 and L-1630 and of reciprocal F_1s at the seed and plant level from their crosses

Parent of cross	C22:1 (%)		
	Parent	F_1 seed	F_1 plant (F_2 seeds averaged)
L-2890	8.2 ± 1.3 a[a]		
C-101	45.1 ± 2.5 c		
L-2890 × C-101		39.2 ± 0.9 b	37.9 ± 10.2 a
C-101 × L-2890		40.1 ± 0.9 b	37.5 ± 9.7 a
L-2890	10.9 ± 0.7 a[a]		
L-1630	54.8 ± 2.3 c		
L-2890 × L-1630		44.2 ± 1.6 b	38.1 ± 9.34 a
L-1630 × L-2890		46.5 ± 1.6 b	39.8 ± 8.36 a
L-1630	53.3 ± 3.4 a[a]		
L-25X-1	0.02 ± 0.13 c		
L-1630 × L-25X-1		34.2 ± 1.8 b	30.3 ± 12.8 a
L-25X-1 × L-1630		36.2 ± 1.6 b	30.4 ± 13.3 a
L-2890	9.9 ± 1.1 a[a]		
L-25X-1	0.04 ± 0.1 c		
L-2890 × L-25X-1		29.3 ± 2.4 b	22.4 ± 11.2 a
L-25X-1 × L-2890		27.6 ± 4.0 b	23.2 ± 11.7 a

[a] Values followed by the same letter are not significantly different at the 0.05 probability level based on *t*-tests

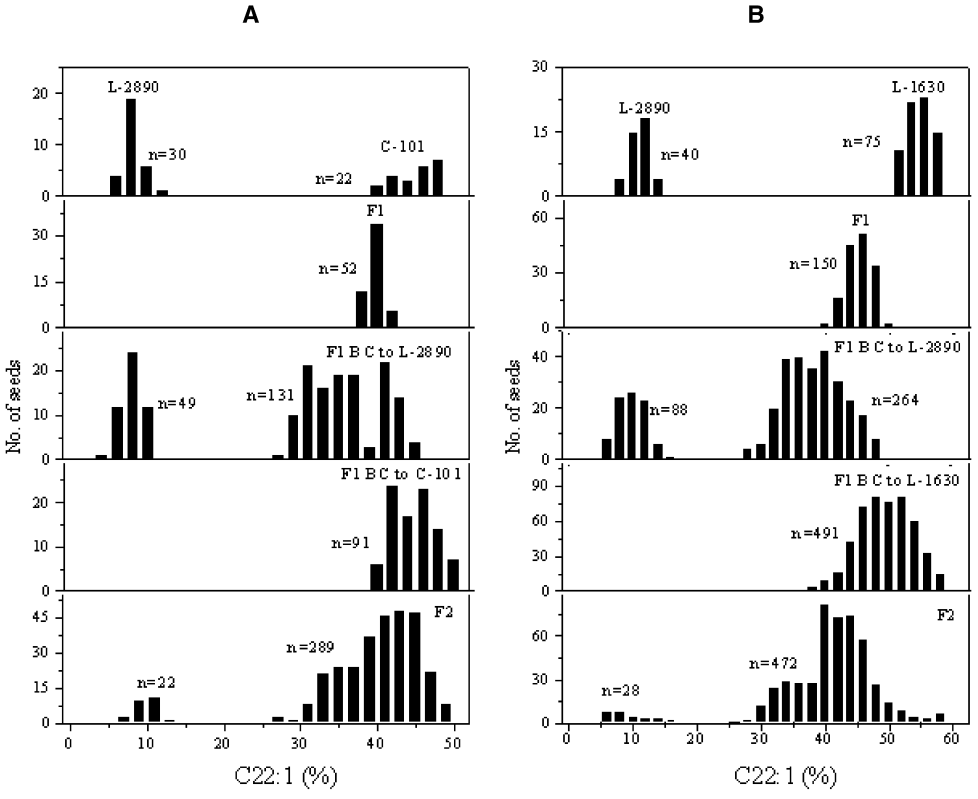

Fig. 1 Frequency distributions of erucic acid content in individual seeds of: **A** L-2890 (P_1), C-101 (P_2), and their F_1 (reciprocal F_1 s pooled), F_2 (five F_2 populations pooled), BC_1 to P_1 (four BC populations pooled) and BC_1 to P_2 (two BC populations pooled) generations; **B** L-2890 (P_1), L-1630 (P_2), and their F_1 (reciprocal F_1s pooled), F_2 (six F_2 populations pooled), BC_1 to P_1 (nine BC populations pooled) and BC_1 to P_2 (eight BC populations pooled) generations. n = number of individuals in each group or class

observed for L-2890, and the second class to the combined category "intermediate-high" with a C22:1 range between 26.7 and 49.5% in L-2890 × C-101 and 26.6 and 58.6% in L-2890 × L-1630. In both crosses the observed data (Fig. 1A, B) satisfactorily fit a phenotypic ratio of 1 low: 15 intermediate-high ($\chi^2 = 0.36$, $P = 0.55$ and 0.38, $P = 0.54$). These data indicate that the C22:1 content in mutant line L-2890 is controlled by two independent loci (tentatively designated E_x and E_y) with partial dominance of high over low C22:1 content (L-2890 genotype: $e_x e_x e_y e_y$). The backcrosses to both parents were analysed for L-2890 × C-101 and L-2890 × L-1630 crosses. According to the two-gene model, a ratio of 1:3 (low:intermediate + high) was to be expected in the backcrosses to the low C22:1 parent line L-2890, whereas all the individuals were expected to be in the high class in the backcross to the high parents, C-101 and L-1630. The data observed (Fig. 1A, B), fitted the theoretical ratio 1:3 in the backcross to L-2890 for L-2890 × C-101 ($\chi^2 = 0.56$, $P = 0.75$) and for L-2890 × L-1630 ($\chi^2 = 0.0$, $P = 1$), thus supporting the proposed model.

This genetic system, of two genes determining C22:1 content, with almost complete dominance of high over low C22:1 content, differ from the model of two unlinked genes acting in an additive manner, described in crosses between zero and high C22:1 in the amphiploid *Brassica* species *B. napus* (Harvey and Downey 1964), *B. juncea* (Kirk and Hurlstone 1983) and in *B. carinata* [(Getinet et al. 1997), in the present work (see cross L-1630 × L-25X-1 described below)]. The same model, digenic control and additive action, has been reported by Alemayehu and Becker (2001) in crosses involving low C22:1 lines in *B. carinata*. However, Chen and Heenen (1989) described a genetic system of two partially dominant genes for the control of C22:1 content in crosses of a re-synthesized high-erucic *B. napus* line with a zero-erucic cultivated line. The results of the segregation in the cross between the mutant L-2890 and its parental line C-101 indicate that two simultaneous single-gene mutations were induced at two different unlinked loci. Induced mutants with altered fatty acid profiles have usually been the result of a mutation at a single locus, although two simultaneous mutations at different loci have also been reported (Velasco et al. 1999).

Genetic control of zero (<0.5%) C22:1 content in the interspecific derived line L-25X-1 in crosses with high erucic acid line L-1630 (55%)

The mean C22:1 content of F_1 seeds of the cross between the high C22:1 line L-25X-1 × L-1630 was intermediate between both parents, but with a small shift towards the levels of the high erucic parent L-1630 (Table 2, Fig. 2). The reciprocal F_1s did not differ significantly for the C22:1 content (Table 2), indicating the absence of maternal effects as concluded above in the crosses with L-2890 and confirming previous genetic studies of zero C22:1 in *B. carinata* (Getinet et al. 1997). The C22:1 content of individual seeds from the BC_1F_1 backcross to the zero C22:1 parent L-25X-1 ranged from zero to 37% showing three clear-cut classes (Fig. 2). The first class

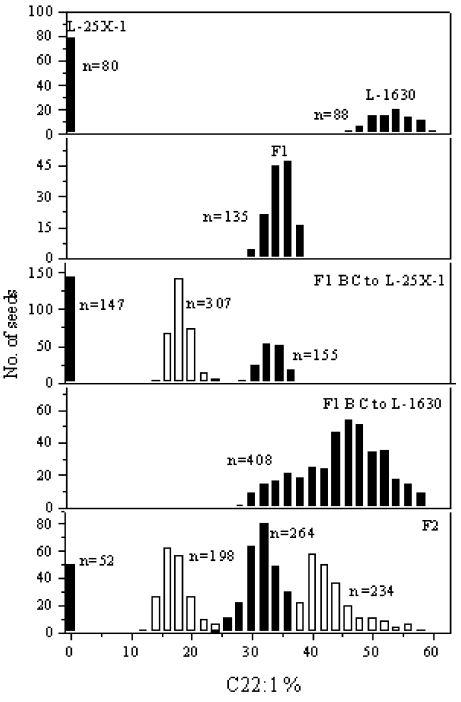

Fig. 2 Frequency distributions of erucic acid content in individual seeds of L-1630, L-25X-1 (P_1), L-1630 (P_2), and their F_1 (reciprocal F_1s pooled), F_2 (six F_2 populations pooled), BC_1 to P_1 (12 BC populations pooled) and BC_1 to P_2 (12 BC populations pooled) generations. *Empty and dark vertical bars* represent different erucic acid classes. n = number of individuals in each group or class

had similar C22:1 values, <0.5%, to the zero C22:1 parent L-25X-1. The second class ranged from 10 to 20% which was greater than the content of L-25X-1 and smaller than the lower limit of the F_1 generation. The third class ranged from 27% to 37%, similar to the range shown by the F_1 generation. The data observed for the three classes (Fig. 2) fit a phenotypic ratio 1:2:1 ($\chi^2 = 0.25$, $P = 0.88$) which represents a two-gene model with additive effects. The backcross to the high C22:1 line, L-1630, ranged between 29 and 56% (Fig. 2) which was similar to the range between the lower limit of the F_1 seeds and the upper limit of L-1630. The frequency distribution did not show any division of phenotypic classes and no theoretical ratio was tested.

The C22:1 content of the F_2 seeds ranged from 0 to 58.9% showing a discontinuous distribution with four classes apparent (Fig. 2). Three of these classes with C22:1 ranging between 0–0.5%, 10–27% and >27–37% respectively, corresponded to those described above in the backcross to L-25X-1, whereas an additional class ranging between 37%, the upper limit of the F_1, and the upper limit of L-1630, was novel. The ratio observed for the four classes defined (Fig. 2) fitted a ratio 1:4:6:5 ($\chi^2 = 2.20$, $P = 0.53$). This segregation confirms the two-gene genetic model with additive effects and an equal action of alleles at both loci. The proposed genotypes for the lines used in this cross were $e_1e_1e_2e_2$ for the zero C22:1 line L-25X-1 and $E_1E_1E_2E_2$ for the high C22:1 line L-1630. This model of digenic control of erucic acid content has been described previously in *B. carinata* (Getinet et al. 1997) and in other amphidiploid *Brassica* species (Harvey and Downey 1964; Kirk and Hulstone 1983).

Krzymanski and Downey (1969) and Anand and Downey (1981) established that there are at least five alleles governing the levels of C22:1 in the seed oils of *B. napus* and *B. rapa*. These alleles were depicted by the symbols *e*, E^a, E^b, E^c and E^d, with each controlling the synthesis of 0, 10, 15, 30 and 3.5% respectively. In the present study, taking into account the C22:1 of L-1630 (about 55%), each of the proposed alleles, E_1 or E_2, would be responsible for an increase of approximately 14 percentage points in C22:1. It could well be that the alleles E_1 and E_2 contributing about 14% C22:1 in the line L-1630 are the same as the E^b allele. In this study the cross of C-101, with 40–45% C22:1, with the zero C22:1 line, was not included. However, Getinet et al. (1997) crossing a zero C22:1 *B. carinata* line with a cultivar with a similar C22:1 content like C-101, concluded that alleles contributing about 10% C22:1, with the same strength as the E^a alleles, were involved. Further studies are needed to clarify if L-1680 and C-101 carry alleles with a different contribution of C22:1 at the loci *E1* and *E2*, or if minor genes are responsible for the different C22:1 content in these lines.

Genetic relationships between low (10%) and zero (<0.5%) C22:1 concentrations in crosses between L-2890 and L-25X-1

The C22:1 content in the seeds of L-2890, and L-25X-1 lines and their reciprocal F_1 seeds and reciprocal F_1 plants, is shown in Table 2. The reciprocal F_1 seeds and F_1 plants did not differ significantly for C22:1 content, indicating the absence of maternal and cytoplasmic effects. The average C22:1 content in reciprocal F_1 seeds (28.4%) was much higher than the mid-parent value (5.0%) and different from both parents, L-2890 (9.9%) and 25X-1 (0.04%) (Table 2, Fig. 3). A strong transgressive segregation was also observed in the segregating F_2 and BC_1F_1 generations (Fig. 3). The C22:1 content of individual F_2 seeds ranged from 0% to 45.7%, the BC_1F_1 to L-2890 from 1.7 to 47.3%, and the BC_1F_1 to L-25X-1 from 0 to 35.1%. The transgressive segregation found for the C22:1 content in the F_1 and segregating generations indicates that the alleles determining the low C22:1 in line L-2890, temporarily designated e_x and e_y (see above), are located at different loci than the zero C22:1 alleles e_1 and e_2 in line L-25X-1. The loci *E1* and *E2* are probably the same as those described by Getinet et al. (1997) in *B. carinata*. The partially recessive alleles, e_x and e_y, determining the low C22:1 in L-2890 were induced after mutagenic treatment. We propose the designation of m_1 and m_2 for these alleles, and M_1 and M_2 for the loci involved in the control of C22:1 in L-2890 to distinguish them from the classical denomination of recessive *e* alleles for zero C22:1 at two loci described in amphidiploid *Brassica* species (Harvey and Downey 1964; Kirk and Hurlstone 1983;

Table 3 Proposed genotypes, genotypic frequency and C22:1 phenotypic pattern in the F_2 and BC_1 F_1 generations after crosses between L-25X-1 and the mutant line L-2890. Based on a model of partial dominance at *M1* and *M2* loci, additive effects of *E1* and *E2* loci and equal effects of alleles at these loci, which predict genotypic ratios 4:8:4 and 4:12 for BC_1F_1 generations and 16:14:61:165 for the F_2

Generations and proposed genotypes[a]	Genotypic frequency	C22:1 phenotypic pattern
BC_1F_1 to L-25X-1		
eeee M – M –	4	zero (<0.5%)
Eeee M – M –	8	Int. low (>10–20%)
EEee M – M –	4	Int. high (>20–34%)
BC_1F_1 to L-2890		
EE - - mmmm	4	very low + low (1–13%)
EE - - mmMm	8	
EE - - MmMm	4	
	Total: 12	Int. high + high (>20–40%)
F_2		
eeee- - - -	16	zero (<0.5%)
Eeee mmmm	4	
EeEe mmmm	6	
EEEe mmmm	4	
	Total: 14	Very low (1–8.8%)
EEEE mmmm	1	
Eeee M- - -	60	
	Total:61	Low + Int.low (>8.8 – <20%)
EEee M- - -	90	
EEEe M- - -	60	
EEEE M- - -	15	
	Total: 165	Int. high + high (20–45%)

[a] A "-" indicates that any allelic configuration may occur at these loci

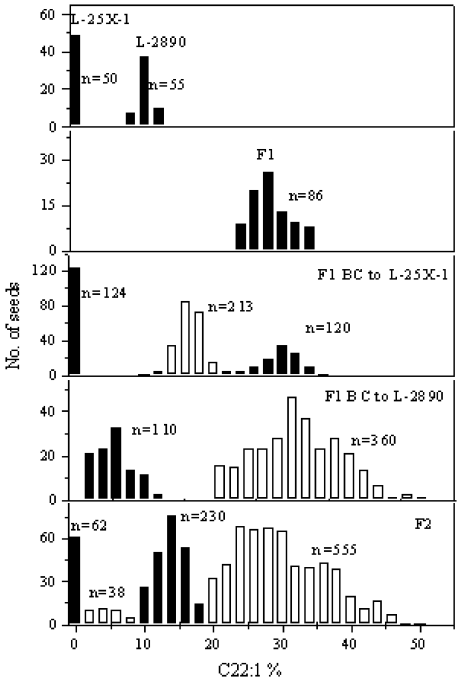

Fig. 3 Frequency distributions of erucic acid content in individual seeds of L-2890, L-25X-1 and their F_1 (reciprocal F_1s pooled), F_2 (six F_2 populations pooled), BC_1 to P_1 (eight BC populations pooled) and BC_1 to P_2 (eight BC populations pooled) generations. *Empty and dark vertical bars* represent different erucic acid classes. n = number of individuals in each group or class

Getinet et al. 1997). Therefore, taking into account the genetic systems proposed above to explain the genetic control of the low, zero and high C22:1 content in crosses L-2890 × C-101, L-2890 × L-1630 and L-25X-1 × L-1630, the genetic constitution of the parent lines would be $E_1E_1E_2E_2m_1m_1m_2m_2$ for L-2890, $e_1e_1e_2e_2M_1M_1M_2M_2$ for L-25X-1 and $E_1E_1E_2E_2M_1M_1M_2M_2$ for C-101. Assuming equal effects of alleles *E* at *E1* and *E2* loci, and of alleles *M* at *M1* and *M2* loci, these genotypes can be expressed as *EEEEmmmm*, *eeeeMMMM* and *EEEEMMMM* respectively. The genetic model proposed, consisting of alleles with additive effects at two loci *E1* and *E2*, and alleles with partial (near complete) dominance at two different loci, *M1* and *M2*, would explain the strong transgressive C22:1 content of F_1 seeds (genotype *EeEeMmMm*) which showed much higher mean values than L-2890 (Table 2, Fig. 3). On the basis of this genetic model the expected F_2 and BC_1F_1 ratios were calculated by grouping the different genotypes into phenotypic classes. Table 3 shows the possible allelic configurations of F_2 and BC_1F_1 generations and their phenotypic expression.

The seeds of the backcross to the zero C22:1 parent L-25X-1 were analysed. Three clear-cut C22:1 classes ranging between 0–0.5% (zero), 10.0–20.0% (intermediate low) and 20.0–34.0% (intermediate high) were distinguished (Fig. 3). The observed data for these classes (Fig. 3) satisfactorily fitted a phenotypic ratio of 4:8:4 (χ^2 = 2.17, P = 0.34). This ratio was predicted by grouping the genotypic classes on the basis of dominance of *M* alleles at the *M1* and *M2* loci, and additive effects of *E* alleles at the *E1* and *E2* loci (Table 3). The genotypes expected for these phenotypic classes were: 4 *eeeeM-M-*, 8 *EeeeM-M-* and 4 *EEeeM-M* -, where "-" indicate that any allelic configuration may occur at these loci.

In the eight backcrosses to the low C22:1 parent line L-2890, combined in Fig. 3, two clear-cut classes were distinguished (Fig. 3), one, ranging from 1.7 to 11.1%, including "very low" + "low" (≤L-2890) C22:1 phenotypes, and the second, including individuals with "intermediate high" + "high" C22:1 content (range 20.0 to

43.7%). The frequencies observed in each class (Fig. 3) satisfactorily fitted a 4:12 ratio ($\chi^2 = 0.63$, $P = 0.42$). This segregation would be the expected one according to the four-loci model proposed (Table 3). The occurrence of segregant phenotypes with C22:1 values under the lowest C22:1 limit of L-2890 (*EEEEmmmm*) was expected for genotypes with recessive alleles at both loci *M1* and *M2* and carrying one or two *e* alleles (genotypes *EEeemmmm* and *EEEemmmm*) (Table 3). Likewise segregants with a high C22:1 content of 43.7%, (genotype*EEEEMmMm*) were observed.

The C22:1 content of the F_2 seeds showed a discontinuous distribution with four peaks (Fig. 3). The C22:1 of the first class ranged from 0 to 0.5% (zero) which was similar to L-25X-1. The second class, with "very low" C22:1 values ranging from 1.0 to (<) 8.8 %, included higher values than in any seed of L-25X-1 and lower than in any seed of L-2890. The third class included individuals with low and intermediate-low C22:1 values ranging from 8.8%, the lower limit of L-2890, to 20.0%, the lower limit of the BC_1F_1 to L-2890. The fourth class included individuals within a wide range of C22:1 values (20.0 to 45.72%) (intermediate-high + high) which followed a continuous distribution and no further division into subclasses was possible. The genetic ratio expected for these classes was calculated by grouping the F_2 genotypic classes into the four phenotypic classes defined above. The genotypes were assigned to each class taking into account the class limits and the phenotypic expression estimated on the basis of dominance at *M1* and *M2* loci, and additive effects at *E1* and *E2* loci (Table 3). The "zero" C22:1 class included genotypes which were homozygous for the *e* alleles and any configuration at *M1* and *M2* loci (*eeee*- - - -). The second class, with "very low" C22:1 values, included recombinant genotypes homozygous for *m* alleles at *M1* and *M2* loci and at least one allele *e* (genotypes *Eeeemmmm, EeEemmmm* and *EEEemmmm*). The third phenotypic class grouped different genotypes with a "low" C22:1 content, such as L-2890 (*EEEEmmmm*), and "intermediate-low" (*EEeeM*- - -).The fourth class included genotypes with C22:1 ranging from about 20% (intermediate-high; *EeeeMmmm*) to high (over 45%; *EEEEMMMM*). With this grouping the genotypic ratio expected for the classes zero, very low, low + intermediate-low and intermediate-high + high was calculated to be: 16:14:61:165. The number of seeds observed in each class was 62:38:230:555 (Fig. 3). This observed ratio satisfactorily fitted the expected ratio ($\chi^2 = 5.14$, $P = 0.15$) confirming the proposed four-loci model.

As a further confirmation of the genetic model proposed a progeny test was conducted on crosses between L-25X-1 and L-2890 by analysing the F_3 seeds from each of nine F_2 plants. These plants were selected on the basis of the C22:1 content of the corresponding F_2 half-seeds which covered the whole range of C22:1 levels observed in the F_2 population. The F_3 families derived from F_2 half-seed plants A-1 and D-4 in the zero C22:1 = 0.03%, and very high C22:1 = 44.0%, bred true for these characteristics (Fig. 4). The genotype of plant A-1 was identified as being homozygous for *e* alleles at loci *E1* and *E2* (*eeee*- - - -) and the genotype of plant D-4 as being homozygous dominant at the four loci (*EEEEMMMM*).

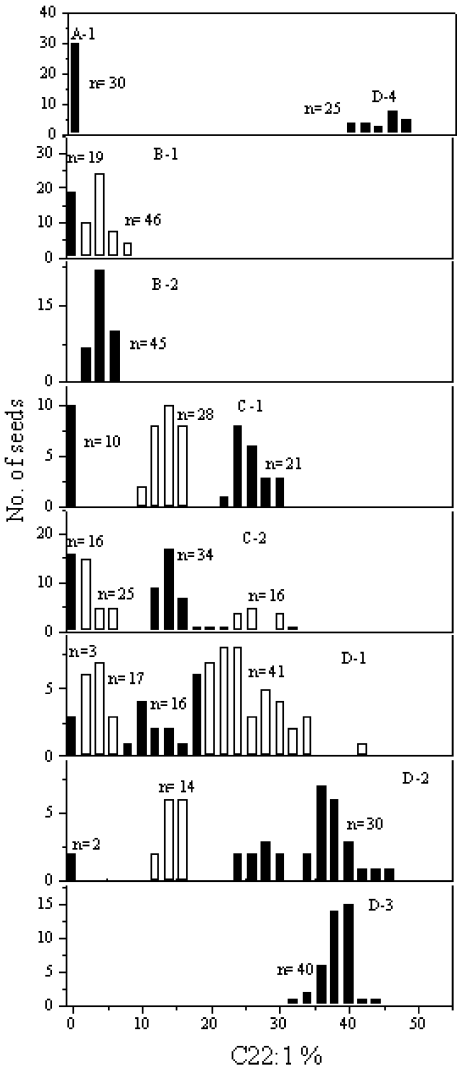

Fig. 4 Frequency distributions of erucic acid content in individual seeds of the F_3 populations from the cross between L-2890 and L-25X-1. *Empty and dark vertical bars* represent different erucic acid classes. n = number of individuals in each group or class

The F_3 seeds from F_2 half-seed plant B-1 containing a very low (2.7%) C22:1, ranged from 0 to about 8% of C22:1. The frequency distribution of C22:1 content was separated into two clear-cut classes (zero:very low) (Fig. 4). The segregation observed for the F_3 seeds of this plant (Fig. 4) satisfactorily fitted the ratio 1:3 (zero:very low) ($\chi^2 = 0.62$, $P = 0.43$) which was expected for the progeny of genotype *Eeeemmmm* (1 *eeeemmmm*:3 *E-eemmmm*). The F_3 seeds of the half-seed plant B-2, also containing a very low (4.2%) C22:1, bred true for "very low" C22:1 values, and was identified as homozygous for *e* alleles at one of the loci, *E1* or *E2*, the other locus being in a homozygous dominant state and recessive homozygosis at loci *M1* and *M2* (genotype *EEeemmmm*).

The F_3 seeds from F_2 half-seed plants C-1 and C-2 from the "low + intermediate low" phenotypic class (C22:1 content >8.8 to <20%) showed two different behaviors (Fig. 4). The C22:1 content of F_3 seeds from plant C-1 ranged from 0 to 31.46% and the frequency distribution of C22:1 content was separated into three clear-cut classes (zero:intermediate-low:intermediate-high). The observed numbers for these classes (Fig. 4) fitted a ratio of 1:2:1 ($\chi^2 = 4.25$, $P = 0.12$). The absence of individuals in the very low class (1 to 8.8% C22:1) and of individuals with very high C22:1 values (>40%) indicated segregation at one of the loci *E1* or *E2*, the other being a homozygote for alleles *e*, and at least one of the loci *M1* or *M2* being in a homozygous dominant state (genotype *EeeeMM*- -). The expected genotypes and frequency from these F_2 plants would be: 1 *eeeeMM*- -, 2 *EeeeMM*- - and 1 *EEeeMM*- -. The C22:1 content of F_3 seeds from the half-seed plant C-2 showed a similar C22:1 range to that of plant C-1 indicating homozygosis for *e* alleles at one of the loci *E1* or *E2* as in plant C-1. However, the presence of individuals in the very low (1–8.8%) C22:1 class, not present in seeds of plant C-1, indicated recessive homozygosis for one of the loci *M1* or *M2* and heterozygosis for the other. Therefore, the proposed genotype for this plant was *EeeeMmmm*. The segregation observed for the F_3 seeds of this plant (Fig. 4) satisfactorily fitted the ratio 4:3:6:3 (zero:very low:low + intermediate-low:intermediate-high + high) ($\chi^2 = 5.76$, $P = 0.12$) expected for the progeny of this genotype (4 *eeee*- - - -:3 *E-eemmmm*:6 *EeeeM-mm*:3 *EEeeM-mm*).

The range for the C22:1 content in the F_3 seeds from F_2 half-seed plants D-1 and D-2 arising from the "Intermediate-high + high" F_2 phenotypic class (C22:1 of F_2 half-seeds 20.5 and 27.2, respectively) showed in both cases a wide C22:1 range from 0 to about 45% (Fig. 4). The F_3 seeds from the half-seed plant D-1 segregated for the four C22:1 phenotypic classes:zero, very low, low + intermediate-low and intermediate-high + high (Fig. 4), fitting a 4:15:12:33 ratio ($\chi^2 = 2.63$, $P = 0.45$) which was expected for the progeny of the F_2 genotype *EeEeMmmm* (4 *eeee*- - - -:15 *E*- - -*mmmm*:12 *EeeeM-mm*:33- -*EE–M-mm*). In contrast, the F_3 seeds from plant D-2 did not show any plant in the very low C22:1 class indicating that at least one of the loci *M1* or *M2* was in a homozygous dominant state. The segregation of this plant (Fig. 4) fitted a 1:4:11 (zero:low + intermediate-low:intermediate-high + high) ratio ($\chi^2 = 0.26$, $P = 0.64$) expected for the progeny of a F_2 genotype *EeEeMM*- -:1 *eeee*- - - -:4 *EeeeMM*- -:11 *EE--MM*- -. Finally, the F_3 seeds from plant D-3 (C22:1 = 38.1%) showed a continuous distribution for their C22:1 content ranging from 31.6 to 43.6% (Fig. 4) which was interpreted in terms of a homozygosis dominant at loci *E1* and *E2*, and one of the loci *M1* or *M2* and heterozygosis for the other (genotype *EEEEMMMm*).

The results of the present study contrast with previous studies on the genetic relationship between genetic systems controlling zero and low C22:1 levels in *B. napus* (Krzymanski and Downey, 1969) which reported a lack of transgressive C22:1 values indicating that the traits were controlled by alleles in the same loci. In the present study, the existence of different loci for the low and zero C22:1 in lines L-2890 and L-25X-1 could be attributed to the origin of these traits. In the case of the low C22:1 mutant L-2890, the mutagenic treatment only induced the partially recessive mutations in the wild alleles *M1* and *M2*, reducing the levels of C22:1, without affecting the wild alleles *E1* and *E2*. Conversely, the zero C22:1 line L-25X-1 obtained by interspecific crossing of *B. carinata* C-101 and *B. napus*, and the *B. juncea* zero C22:1 non-induced mutants (Fernández-Martinez et al. 2001), carried the wild alleles *M* at loci *M1* and *M2* and alleles *e* for zero C22:1 concentration at loci *E1* and *E2*. Recent molecular and biochemical studies in rapeseed, an Ethiopian mustard's close relative, have shown that the zero C22:1 concentration is associated with an absence of β-ketoacyl-CoA (KCS) activity. (Roscoe et al. 2001). KCS, the enzyme which catalyses the elongation pathway of oleic to erucic acid, is encoded by *FAE1* genes, which have been shown to be tightly linked to the *E1* and *E2* loci controlling the C22:1 content in rapeseed (Barret et al. 1998; Fourmann et al. 1998). Our hypothesis is that the zero C22:1 line L-25X-1 has mutations in the *E1* and *E2* genes encoding for the KCS enzyme, while the low C22:1 line L-2890 carries recessive alleles m_1 and m_2 which modify the expression of these genes. Further biochemical and molecular studies are needed to validate this hypothesis.

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