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Development of high-oleic, low-linolenic acid Ethiopian-mustard (*Brassica carinata*) germplasm

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Abstract Seed oil of current zero erucic-acid germplasm of Ethiopian mustard (*Brassica carinata* A. Braun) is characterized by a low concentration of oleic acid and high concentrations of linoleic and linolenic acids. Sources of increased oleic-acid (HO) and reduced linolenic-acid (LL) concentration have been developed separately in high erucic-acid germplasm. The objectives of the present research were to study the inheritance of the HO and LL traits in crosses HO × LL, and to develop HOLL recombinants, both in high erucic-acid and zero erucic-acid backgrounds. The HO mutant N2-3591 (about 20% oleic acid compared to 9% in conventional high erucic-acid materials), was reciprocally crossed with the LL lines N2-4961 and HF-186 (both with about 5% linolenic acid compared to 12% in standard high erucic-acid materials). Increased oleic acid concentration of N2-3591 was found to be controlled by alleles at one locus (Ol), whereas three different loci for reduced linolenic-acid concentration (Ln, Ln1 and Ln2) were identified in N2-4961 and HF-186. Crosses between N2-3591 and N2-4961 generated HOLL recombinants where levels of increased oleic-acid and reduced linolenic-acid were similar to those of the parents. However, a transgressive segregation for oleic acid was observed in crosses between N2-3591 and HF-186, where F_2 seeds with up to 29.7% oleic acid were obtained, in comparison to an upper limit of 25.1% in the N2-3591 parent grown in the same environment. The transgressive increased oleic-acid was expressed in the F_3 generation and was attributed to the presence of a second locus, designated Ol2. The transgressive trait was transferred to the zero erucic-acid line 25X-1, resulting in a zero erucic-acid germplasm with very high oleic-acid concentration (83.9% compared to 32.9% in 25X-1) and low linolenic-acid concentration (5.0% compared to 16% in 25X-1). Additionally, two other lines exhibiting different stable levels of increased oleic-acid (70.7% and 79.5%, respectively) and reduced levels of linolenic-acid (7.5% and 8.7%, respectively) were isolated.

Keywords *Brassica carinata* · Ethiopian mustard · Fatty acids · High oleic-acid · Low linolenic-acid

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Introduction

Autoxidation is the principal cause of oil quality deterioration during processing and storage (Kamal-Eldin and Appelqvist 1996). Polyunsaturated fatty acids are particularly susceptible to this process. The double bonds present in polyunsaturated fatty acids are the active sites that react with oxygen, resulting in oxidation products that are a major source of off-flavors and odors in the oil (Tatum and Chow 1992).

In comparison to canola (zero erucic, low glucosinolate-cultivars of *Brassica* spp.) oil types, present forms of zero erucic-acid Ethiopian mustard (*Brassica carinata* A. Braun) are characterized by a seed oil relatively low in oleic acid (mono-unsaturated) and rich in linoleic and linolenic acids (poly-unsaturated). The oil profile of zero erucic-acid Ethiopian mustard consists of 33% oleic, 37% linoleic and 21% linolenic acid (Alonso et al. 1991; Getinet et al. 1994; Fernández-Martínez et al. 2001), compared to 61% oleic-, 21% linoleic- and 11% linolenic-acid in canola (Scarth and McVetty 1999). Additionally, high oleic-acid (>80%) germplasm has been developed in *Brassica napus* (Wong and Swanson 1991; Auld et al. 1992; Rücker and Röbbelen 1997; Stoutjesdijk et al. 1999) and *Brassica rapa* (Auld et al. 1992; Tanhuanpää et al. 1996). Increased levels of oleic acid (>70%) have been reported also in *Brassica juncea* (Stoutjesdijk et al. 1999). The increase of oleic-acid concentration and the simultaneous reduction of polyunsaturated fatty acid levels are important breeding-objectives in Ethiopian mustard.

Sources of increased oleic-acid and reduced linolenic-acid concentration have been developed in high erucic-acid Ethiopian mustard. Velasco et al. (1997a) obtained three mutants (N2-1992, N2-3591 and N2-6285) with a twofold increase in oleic-acid concentration (about 20% compared to 10% in the control) and a mutant (N2-4961) with linolenic-acid concentration reduced by half (about 6% compared to 12% in the control). A second source with similar reduced levels of linolenic acid (HF-186) was developed by Velasco et al. (1997b) through selection from a germplasm accession of this species. Genetic studies on this material revealed that the increased oleic-acid concentration in N2-3591 was determined by partially recessive alleles at the Ol locus (Velasco et al. 2003), whereas two different loci were identified for reduced linolenic-acid concentration, Ln in N2-4961 (Velasco et al. 2002) and Ln1 in HF-186 (unpublished data).

The objectives of the present research were (1) to study the inheritance of increased oleic-acid and reduced linolenic-acid concentration from crosses of N2-3591 with N2-4961 and HF-186, and (2) to develop recombinants with increased oleic-acid and reduced linolenic-acid concentration, both in high erucic-acid and zero erucic-acid backgrounds.

Materials and methods

Plant material

The Ethiopian mustard germplasm used in this study were the mutant N2-3591, with high erucic-acid and increased oleic-acid concentration, and the mutant N2-4961, with high erucic-acid and reduced linolenic-acid concentration, developed from the line C-101 by mutagenesis (Velasco et al. 1997a); the line HF-186, with high erucic-acid and reduced linolenic-acid concentration, was developed by selection from a germplasm accession (Velasco et al. 1997b); and the zero erucic-acid line 25X-1, was developed from interspecific crosses with *B. juncea* (L.) Czern. and *B. napus* L. (Fernández-Martínez et al. 2001). The average seed-oil fatty acid composition of these lines and the line C-101, of standard seed-oil fatty acid profile, is presented in Table 1.

Crossing and selection scheme

Single seeds of N2-3591, N2-4961 and HF-186 were analysed for the fatty acid composition of the oil by the half-seed method and derived plants grown in the greenhouse in 1997. Plants of N2-3591 were reciprocally crossed with plants of N2-4961 and HF-186. In all cases, plastic bags were used to prevent cross-pollination.

F_1 half-seeds from reciprocal crosses as well as seeds from the parents were analysed for fatty acid composition and the corresponding plants grown in the greenhouse in 1998. F_1 plants from reciprocal crosses were self-pollinated to obtain F_2 seeds and also backcrossed to both parents. Reciprocal crosses were repeated in order to obtain F_1 seeds under the same environment as the F_2 and BC_1 seeds.

Random seed samples of the parents, F_1, F_2, and BC_1 generations were analysed for fatty acid composition. Since all generations were grown in the same environment, the fatty acid composition of the parents was used to make the parental classifications. Limits of the parental classes were defined as a mean of the parent ±2 standard deviations. Evaluation of fatty acid composition at the F_1 plant level was performed by averaging the fatty acid composition of F_2 seeds from each F_1 plant. The chi-square test was used to evaluate proposed segregation ratios. Reciprocal F_1 means were compared using independent *t*-tests.

An F_2 transgressive recombinant plant with higher oleic-acid concentration than N2-3591, identified in crosses between N2-3591 and HF-186, was crossed with a plant of the zero erucic-acid line 25X-1 in 1999, with the objective of developing zero erucic-, high oleic-, and low linolenic-acid Ethiopian mustard. F_1 half seeds were analysed for fatty acid composition and the corresponding plants were grown in the greenhouse in 2000 and self-pollinated. As F_2 seeds were expected to segregate widely for fatty acid profile, a preliminary screening to identify zero erucic-acid F_2 seeds was conducted by near-infrared reflectance spectroscopy (NIRS) (Velasco et al. 1999). Putative zero erucic-acid half-seeds were analysed for fatty acid composition by gas-liquid chromatography (GLC), and recombinant F_2 plants were grown in the greenhouse in 2001. F_3 half-seeds were analysed by GLC, and F_3 plants were grown in the greenhouse in 2002. F_4 seeds were analysed by GLC to confirm the zero erucic-, high oleic-, low linolenic-acid phenotype of the selected materials. Plants of N2-3591, HF-186 and 25X-1 were grown as checks in all generations.

Analysis of fatty acid composition by gas-liquid chromatography

The fatty acid composition of the seed oil was determined by simultaneous oil extraction and methyl esterification (Garcés and Mancha 1993) followed by gas-liquid chromatography (GLC) of fatty acid methyl esters on a Perkin-Elmer Autosystem gas-liquid chromatograph (Perkin-Elmer Corporation, Norwalk, Conn.) equipped with a 2 m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, Pa.). A temperature program of 190 °C for 10 min, increasing 2 °C min^{-1} up to 220 °C was used. The injector and flame-ionization detector were held at 275 and 250 °C, respectively.

Screening for fatty acid composition by near-infrared reflectance spectroscopy

Single Ethiopian mustard seeds were non-destructively scanned by NIRS on a monochromator NIRSystems model 6500 (NIRSystems, Inc., Silver Spring, Md.). The standard ring cup (IH-0307, Infrasoft

Table 1 Mean and standard deviation of major fatty acids (expressed in % of the total fatty acids in the seed oil) of the Ethiopian mustard lines C-101, N2-3591, N2-4961, HF-186 and 25X-1[a]

Line	16:0[b]	18:0	18:1	18:2	18:3	20:1	22:1
C-101	2.7 ± 0.4	1.1 ± 0.3	9.0 ± 1.1	19.6 ± 1.3	12.2 ± 0.7	6.7 ± 0.5	45.6 ± 1.8
N2-3591	2.5 ± 0.1	0.7 ± 0.1	20.4 ± 2.2	6.1 ± 0.5	11.7 ± 1.4	11.0 ± 1.1	47.0 ± 1.7
N2-4961	3.2 ± 0.2	0.9 ± 0.2	10.9 ± 0.8	23.0 ± 0.7	4.7 ± 0.7	9.1 ± 0.8	44.7 ± 0.8
HF-186	2.9 ± 0.3	0.5 ± 0.1	8.6 ± 1.0	21.9 ± 0.8	4.9 ± 0.6	6.0 ± 0.5	52.3 ± 1.7
25X-1	5.3 ± 0.5	1.3 ± 0.2	32.9 ± 3.5	42.6 ± 2.3	16.4 ± 2.4	1.2 ± 0.2	0.1 ± 0.0

[a] Data based on 16 to 24 plants per line grown under greenhouse conditions
[b] 16:0 = palmitic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = linolenic acid, 20:1 = eicosenoic acid, 22:1 = erucic acid

International, Port Matilda, Pa.) was implemented with an Teflon adapter for single seeds (Velasco et al. 1999).

In a first step, a set of 1,383 Ethiopian mustard seeds with great variability for the fatty acid composition of the oil, chosen from a wide range of breeding materials, were scanned by NIRS and further analysed by GLC in order to develop calibration equations for the major fatty acids. Calibration equations were developed as described by Velasco et al. (1999). The calibration set presented an average fatty acid composition of 25.6% ± 15.4% (mean ± standard deviation) for oleic-acid, 22.6% ± 9.0% for linoleic-acid, 11.3% ± 3.5% for linolenic-acid and 25.8% ± 18.2% for erucic-acid concentration. The standard errors of cross validation (SECV) obtained in the development of the calibration equations were 10.6% for oleic acid, 5.2% for linoleic acid, 2.3% for linolenic acid and 6.8% for erucic acid, resulting in SECV to SD ratios of 0.69 for oleic acid, 0.58 for linoleic acid, 0.65 for linolenic acid and 0.37 for erucic acid. According to Velasco et al. (1999), who developed NIRS calibration equations for estimating the concentration of individual fatty acids in single seeds of rapeseed, the SECV to SD ratios for oleic-, linoleic- and linolenic-acid were too high for the calibration equations to be considered as reliable enough for screening. Consequently, NIRS screening focused on the identification of putative zero erucic-acid F_2 segregants to be further analysed by GLC.

Results

Crosses between N2-3591 and N2-4961

Seeds of N2-3591 had an increased oleic-acid concentration of 21.2% and a standard linolenic-acid concentration of 11.2%. Seeds of N2-4961 had a standard oleic-acid concentration of 10.9% and a reduced linolenic-acid concentration of 4.7% (Fig. 1). Oleic-acid concentration averaged 13.3% in F_1 seeds from the cross N2-3591 × N2-4961, compared to 11.8% in the reciprocal cross, which indicated a partial maternal effect on this trait ($t = 9.12$, $p < 0.01$). The average oleic-acid concentration of the F_1 generation was 12.5%, significantly ($t = 7.50$, $p < 0.01$) lower than the midparent value (16.1%), indicating partial dominance of standard over increased oleic-acid concentration. Linolenic acid averaged 11.4% in F_1 seeds from the cross N2-3591 × N2-4961, compared to 10.4% in the reciprocal cross, which revealed a partial maternal effect on this trait ($t = 8.22$, $p < 0.01$). The average linolenic-acid concentration in F_1 seeds from reciprocal crosses was 10.9%, significantly ($t = 9.98$, $p < 0.01$) higher than the mid-parent value (8.0%), suggesting partial dominance of standard over reduced linolenic-acid concentration.

The average oleic-acid concentration of F_2 seeds was 15.4% in the cross N2-3591 × N2-4961, which was not significantly ($t = 1.45$, $p > 0.05$) different from the average oleic-acid concentration of 15.1% in the reciprocal cross. The average linolenic-acid concentration of F_2 seeds was 9.2% in both crosses. The data revealed the absence of cytoplasmic effects for both fatty acids.

The distribution of F_2 seeds for oleic-acid concentration revealed that approximately one-quarter of the analyzed seeds exhibited the phenotype of N2-3591 (Table 2), indicating the segregation of alleles at a single locus. This was also evident from a 1:1 segregation in BC to N2-3591. Linolenic-acid concentration segregated

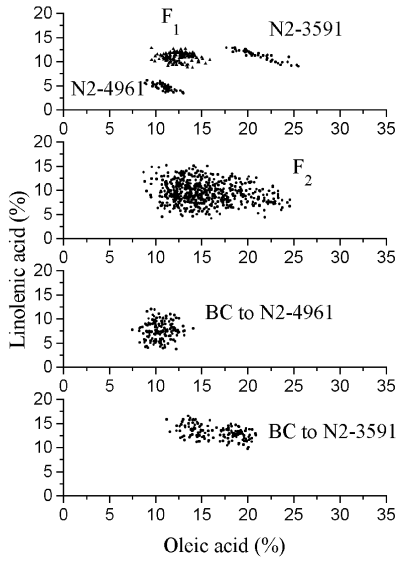

Fig. 1 Scatter plots of oleic- vs linolenic-acid concentration (% of the total fatty acids) in the Ethiopian mustard lines N2-3591 (n = 48), N2-4961 (n = 48) and their F_1 (n = 144), F_2 (n = 573), BC to N2-4961 (n=144) and BC to N2-3591 (n=144) seeds

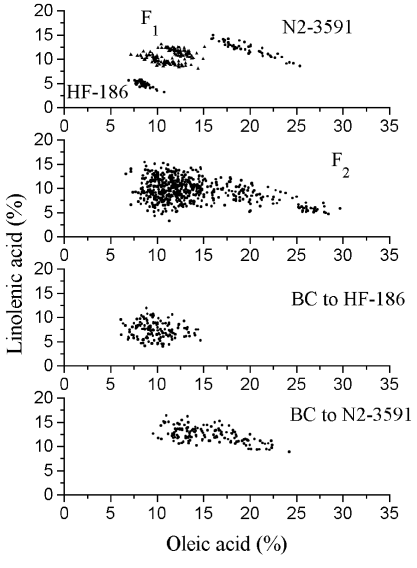

Fig. 2 Scatter plots of oleic- vs linolenic-acid concentration (% of the total fatty acids) in the Ethiopian mustard lines N2-3591 (n = 48), HF-186 (n = 48) and their F_1 (n = 144), F_2 (n = 576), BC to HF-186 (n = 144) and BC to N2-3591 (n = 144) seeds

following 1:15 (=N2-4961:>N2-4961) ratios, suggesting the presence of two loci for this trait (Table 3). This was also evident in the BC to N2-4961, where a 1:3 (=N2-4961:>N2-4961) segregation was found. Five out of 573 F_2 seeds exhibited the phenotype of both parents (>17.2% oleic acid, <6.1% linolenic acid), which is in agreement with the segregation of three independent loci ($\chi^2 = 1.77$, $p = 0.18$).

Table 2 Segregation for oleic-acid concentration in F_2 and BC_1 populations from crosses of the Ethiopian mustard line N2-3591 (increased oleic-, high erucic-acid) with N2-4961 and HF-186 (reduced linolenic-, high erucic-acid)

F_2 or backcross	Number of seeds with oleic acid			χ^2			
	<N2-3591	=N2-3591	>N2-3591	3:1 or 12:3:1	*P*	1:1	*P*
F_2 (N2-3591 × N2-4961)	428	145	0	0.03	0.86		
BC to N2-3591	73	71	0			0.03	0.86
BC to N2-4961	144	0	0				
F_2 (N2-3591 × HF-186)	437	106	33	0.34	0.84		
BC to N2-3591	68	76	0			0.44	0.51
BC to HF-186	144	0	0				

Table 3 Segregation for linolenic-acid concentration in F_2 and BC_1 populations from crosses of the Ethiopian mustard line N2-3591 (increased oleic-, high erucic-acid) with N2-4961 and HF-186 (reduced linolenic-, high erucic-acid)

F_2 or backcross	Number of seeds with linolenic acid		χ^2			
	=N2-4961	>N2-4961	1:15	*P*	1:3	*P*
F_2 (N2-3591 × N2-4961)	34	539	0.10	0.75		
BC to N2-3591	0	144				
BC to N2-4961	34	110			0.15	0.70
F_2 (N2-3591 × HF-186)	41	535	0.74	0.39		
BC to N2-3591	0	144				
BC to HF-186	38	106			0.44	0.51

Crosses between N2-3591 and HF-186

Seeds of N2-3591 had an increased oleic-acid concentration of 19.6% and a standard linolenic-acid concentration of 12.2%. Seeds of HF-186 had a standard oleic-acid concentration of 8.5% and a reduced linolenic-acid concentration of 4.9% (Fig. 2). Oleic-acid concentration averaged 12.4% in F_1 seeds from the cross N2-3591 × HF-186, compared to 10.5% in the reciprocal cross, which indicated a partial maternal effect on oleic-acid concentration ($t = 8.80$, $p > 0.01$). The average oleic-acid concentration in F_1 seeds from reciprocal crosses was 11.5%, significantly ($t = 5.89$, $p < 0.01$) lower than the mid-parent value (14.1%), indicating partial dominance of standard over increased oleic-acid concentration. Linolenic-acid concentration averaged 11.6% in F_1 seeds from the cross N2-3591 × HF-186, compared to 9.6% in the reciprocal cross, which revealed a partial maternal effect on this trait ($t = 15.31$, $p < 0.01$). The average linolenic-acid concentration in F_1 seeds from reciprocal crosses was 10.6%, significantly ($t = 5.54$, $p < 0.01$) higher than the mid-parent value (8.6%), revealing the existence of partial dominance of standard over reduced linolenic-acid concentration.

The average oleic-acid concentration in F_2 seeds from the cross N2-3591 × HF-186 was 13.5%, which was not significantly ($t = 0.23$, $p > 0.05$) different from the average oleic-acid concentration of 13.6% in the reciprocal cross. The average linolenic-acid concentration of F_2 seeds was 9.6% in the cross N2-3591 × HF-186 and 9.4% in the reciprocal cross, which was not significantly ($t = 1.28$, $p > 0.05$) different. Therefore no cytoplasmic effects were detected for oleic- and linolenic-acid concentrations in this cross.

F_2 segregants for oleic-acid concentration clearly surpassed the upper limit of the N2-3591 parent (Fig. 2). The distribution was in accordance with a 12:3:1 (<N2-3591:=N2-3591:>N2-3591) ratio (Table 2), indicating that transgressive segregants were produced by alleles at two loci. The BC to N2-3591 did not produce any transgressive segregant, and the segregation followed a 1:1 (<N2-3591:=N2-3591) ratio. Linolenic acid segregated following 1:15 (=HF-186:>HF-186) ratios, suggesting the presence of two loci for this trait (Table 3). Digenic inheritance for this fatty acid was also found in the BC to HF-186, which exhibited a 1:3 (=HF-186:>HF-186) segregation ratio.

Transgressive F_2 seeds with oleic-acid concentration above the upper limit of N2-3591 showed a significant increase in the oleic- to linoleic-acid ratio compared to the seeds of N2-3591. The average ratio was 3.1 for N2-3591 and 4.7 for transgressive F_2 seeds. The latter were also characterized by a reduced linolenic-acid concentration. The range of variation for linolenic-acid concentration in the whole F_2 population was 3.2 to 15.4%, while it was 4.7 to 8.2% in transgressive F_2 seeds. Twenty five out of 33 transgressive F_2 seeds had a linolenic-acid concentration similar to HF-186. Self-pollinated F_3 seeds, generated from a transgressive F_2, also expressed high oleic- and reduced linolenic-acid traits (Fig. 3).

Transgressive phenotypes had an exceptionally high concentration of total monounsaturated fatty acids in the seed oil. The F_3 generation averaged 31.0% oleic acid (18:1), 7.5% eicosenoic acid (20:1) and 53.4% erucic acid (22:1), which accounted for 91.9% of the total fatty acids in the seed oil, compared to 78.4% in N2-3591 and 61.3% in C-101 (Table 1).

Recombination of increased oleic-/reduced linolenic-acid with zero erucic-acid

The plant derived from a transgressive F_2 seed with increased oleic-acid (29.5%), reduced linolenic-acid (4.2%) and high erucic-acid concentration (50.4%) was crossed with a plant of the zero erucic-acid line 25X-1. F_1

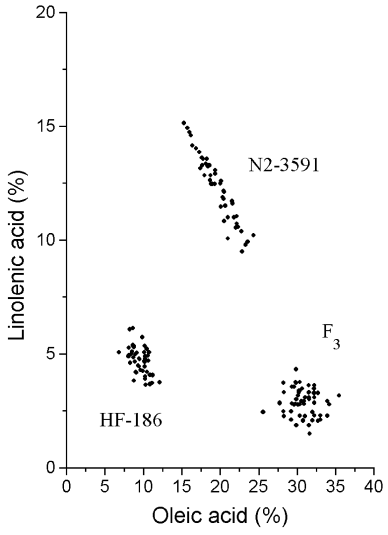

Fig. 3 Scatter plots of oleic- vs linolenic-acid concentration (% of the total fatty acids) in the Ethiopian mustard lines N2-3591 (n = 48), HF-186 (n = 48) and F_3 seeds (n = 60) from transgressive F_2 genotypes

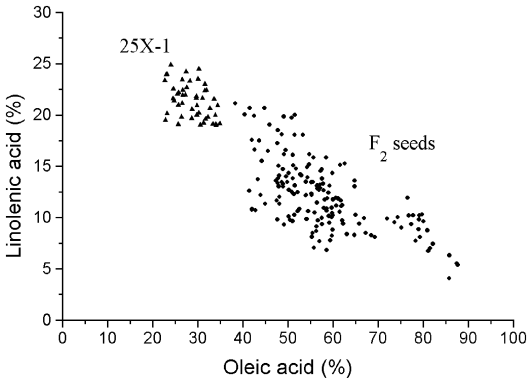

Fig. 4 Scatter plot of oleic- vs linolenic-acid concentration (% of the total fatty acids) in the zero erucic-acid Ethiopian mustard line 25X-1 (n = 48) and the zero erucic-acid F_2 seeds (n = 172) from crosses between HF-186 (reduced linolenic-, high erucic-acid), N2-3591 (increased oleic-, high-erucic-acid) and 25X-1

seeds averaged 28.3% oleic acid, 7.2% linolenic acid and 25.3% erucic acid.

A total of 3,587 F_2 seeds were analysed by NIRS, from where 703 seeds were pre-selected as putative zero erucic-acid phenotypes and were further analysed by GLC. Chromatographic analyses revealed that 172 F_2 seeds were actually free from erucic acid (<1% of the total fatty acids). The oleic- and linolenic-acid concentrations in these zero erucic-acid F_2 seeds are presented in Fig. 4, which also includes the parental line 25X-1 grown in the same environment as the check. From the 172 zero erucic-acid F_2 seeds, 149 seeds had an oleic-acid concentration below 70%, 19 seeds had an oleic-acid concentration between 70% and 82%, and four seeds had an oleic-acid concentration above 85%.

Sixty four zero erucic-acid F_2 seeds covering the range for oleic acid up to 82% were sown and self-pollinated. Most of the F_3 seed families segregated for this trait. However, two F_3 families with different stable levels of increased oleic acid were obtained from F_2 seeds with 67% and 80% oleic acid, respectively. Stable expression of this trait was confirmed in the F_4 generation. Variation for oleic acid in these two lines was 65 to 75% (line AB01169) and 75 to 82% (line AB01066). The average fatty acid composition of both lines is presented in Table 4.

Three of the four F_2 seeds with an oleic-acid concentration above 85% failed to produce mature plants. The fourth seed produced a plant that exhibited strong chlorosis, which was corrected by applying a complete nutritive solution including all essential macro- and micro-elements. F_3 seeds from this plant presented a uniformly high oleic-acid concentration (83.5 to 88.1%) combined with a reduced linolenic-acid concentration (3.7 to 7.5%). These levels were confirmed in the following generation, with F_4 seeds ranging from 82.4 to 86.6% oleic-acid and 3.5 to 6.9% linolenic-acid. This line was designated AB01323 and its average fatty acid composition is presented in Table 4. This high oleic- and low linolenic-acid line suffered from strong chlorosis in the F_3 and F_4 generations also.

Discussion

Crosses of N2-3591 with N2-4961 and HF-186 revealed that the increased oleic-acid concentration in N2-3591 is determined by alleles at a single locus. These results confirm those of Velasco et al. (2003), who reported the segregation of one single gene (Ol) in crosses between N2-3591 and the line C-101, with a normal fatty acid profile.

Two loci for reduced linolenic-acid concentration were segregating in crosses between the increased oleic-acid line N2-3591 and the reduced linolenic-acid lines N2-4961 and HF-186. Previous genetic studies based on

Table 4 Mean and standard deviation of major fatty acids (expressed in % of the total fatty acids in the seed oil) of the zero erucic-acid Ethiopian mustard line 25X-1, with standard fatty acid composition and three $F_{3:4}$ lines with different increased levels of oleic acid

Line	16:0[a]	18:0	18:1	18:2	18:3	20:1
25X-1	5.3 ± 0.5	1.3 ± 0.2	32.9 ± 3.5	42.6 ± 2.3	16.4 ± 2.4	1.2 ± 0.2
AB01169	3.6 ± 0.3	1.6 ± 0.2	70.7 ± 2.4	15.0 ± 1.8	7.5 ± 1.5	1.4 ± 0.1
AB01066	3.8 ± 0.5	1.0 ± 0.3	79.5 ± 1.4	4.8 ± 0.8	8.7 ± 1.3	1.6 ± 0.2
AB01323	3.7 ± 0.6	1.4 ± 0.2	83.9 ± 1.0	4.2 ± 0.4	5.0 ± 0.7	1.5 ± 0.1

[a] 16:0 = palmitic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = linolenic acid, 20:1 = eicosenoic acid

Table 5 Proposed allelic constitutions and average values for oleic- to linoleic-acid ratio and linolenic-acid concentration for the zero erucic-acid Ethiopian mustard line 25X-1, with standard fatty acid composition and three recombinant lines with different increased levels of oleic acid

Line	Oleic/linoleic	Linolenic	Proposed genotype
25X-1	0.8	16.4	*OlOlOl2Ol2LnLnLn1Ln1Ln2Ln2*
AB01169	4.7	7.5	*ololOl2Ol2LnLnln1ln1Ln2Ln2*
AB01066	16.6	8.7	*ololol2ol2LnLn ln1ln1Ln2Ln2*
AB01323	20.0	5.0	*ololol2ol2LnLnln1ln1ln2ln2*

crosses of the reduced linolenic-acid lines with the standard line C-101 indicated monogenic inheritance for reduced linolenic-acid (Velasco et al. 2002). The locus in N2-4961 was designated Ln while that in HF-186 was designated Ln1. The occurrence of digenic segregations in crosses of both lines with N2-3591 suggests the presence of alleles for reduced linolenic-acid concentration at a third locus, which will be tentatively designated Ln2. Recessive alleles at this locus would be present in N2-4961, HF-186 and C-101, but not in N2-3591. Our hypothesis is that the *ln2* alleles have no effect on linolenic-acid concentration in absence of the *ln* or *ln1* alleles. Accordingly, the alleles *ln2* will not have a phenotypic effect for reduced linolenic-acid concentration in C-101, which presents a standard fatty acid profile, thus being feasible different allelic configurations in lines derived from it such as N2-3591 and N2-4961 (Velasco et al. 1997a). Similar models have been postulated for genes controlling increased levels of saturated fatty acids in sunflower (Pérez-Vich et al. 1999a, b). According to this hypothesis, the genetic constitution of the three parents was as follows: *LnLnLn1Ln1Ln2Ln2* for N2-3591, *lnlnLn1Ln1ln2ln2* for N2-4961, and *LnLnln1ln1ln2ln2* for HF-186.

Complex inheritance of reduced linolenic-acid concentration has been described in rapeseed. Classical genetic studies on rapeseed mutants with low linolenic-acid concentration have identified one or two major genes segregating for this trait (Brunklaus-Jung and Röbbelen 1987), whereas molecular studies in the same species have identified one (Hu et al. 1995; Tanhuanpää et al. 1995), two (Jourdren et al. 1996; Thormann et al. 1996; Rajcan et al. 1999) or even three loci (Somers et al. 1998) associated with low linolenic-acid concentration. In spring turnip rape (*B. rapa* ssp. *oleifera*), Tanhuanpää and Schulman (2002) have identified three QTLs affecting linolenic-acid content.

Transgressive oleic-acid concentration was observed in F_2 seeds from crosses between HF-186 and N2-3591, which segregated following 12:3:1 (<N2-3591:=N2-3591:>N2-3591) ratios, but not in those from crosses between N2-4961 and N2-3591. The possibility that the transgressive character resulted from interaction between the *ol* alleles and alleles for reduced linolenic-acid concentration was rejected because not all the transgressive individuals expressed reduced linolenic-acid concentrations. Therefore, the transgressive oleic-acid concentration was attributed to the presence of alleles affecting oleic-acid concentration at a second locus, tentatively named Ol2. It is hypothesized that the *ol2* alleles are present in HF-186 and that they have no phenotypic effect on oleic-acid content in the absence of *ol* alleles. The proposed genotypic constitutions of the three parents used in this study are *OlOlOl2Ol2* for N2-4961, *OlOlol2ol2* for HF-186, and *ololOl2Ol2* for N2-3591.

The existence of the Ol2 locus explains the differences between the recombinant lines AB01169 (70.7% oleic, 15.0% linoleic, 7.5% linolenic acid) and AB01066 (79.5% oleic, 4.8% linoleic, 8.7% linolenic acid). Both lines present similar levels of linolenic-acid and different oleic- to linoleic-acid ratios (4.7 for AB01169 and 16.6 for AB01066), which is attributed to the effect of *ol2* alleles. The line AB01323 has an oleic- to linoleic-acid ratio (20.0) slightly higher than AB01066 but a lower linolenic-acid concentration (Table 4). Therefore it is hypothesized that both lines are different for *ln2* alleles. Proposed genotypes of the three lines with increased oleic-acid concentration as well as the standard line 25X-1 are presented in Table 5.

Oils with high levels of oleic acid in combination with reduced levels of polyunsaturated fatty acids show a higher oxidative stability, which is associated with longer shelf-life and dietary benefits (McVetty and Scarth 2002). No material with high levels of oleic acid has been reported so far in *B. carinata*. In the present research, germplasm of *B. carinata* with three different levels of increased oleic-acid concentration has been developed, exhibiting ranges of variation for oleic-acid concentration from about 65 to 75%, 75 to 82% and 82 to 87%, respectively. Different food and industrial applications require different oleic-acid levels. Whereas very high oleic-acid levels (>85%) are optimal for food and industrial applications requiring very high oxidative stability (Johnson 1999), mid-oleic oils (67 to 75%) are preferred for applications which require high cooking and frying temperature, and for snack foods requiring long shelf-life (McVetty and Scarth 2002).

Germplasm with high oleic-acid concentration in a high erucic-acid background has also been developed. This germplasm is characterized by a seed oil with about 92% monounsaturated fatty acids in the seed oil, distributed as 31% oleic acid, 8% eicosenoic acid and 53% erucic acid. To our knowledge, these are the highest levels of mono-unsaturated fatty acids reported so far in high erucic materials of *Brassica* spp. Current high erucic-acid oils are industrial feedstocks with a broad range of applications, including lubricants, emollients,

fuels, polymers, cosmetics and pharmaceuticals (Sonntag 1995). Some of these applications might benefit from the additional presence of increased levels of oleic acid in the high erucic-acid oil.

The genotypes with very high oleic-acid concentration (>82%) were partially chlorotic, which was found to be heritable. Although such a negative trait could be prevented during the course of the present research by application of a complete nutritive solution at different stages of development, genetic and physiological investigations to characterize the basis of such chlorosis are underway.

In summary, the present research revealed the presence of two loci for increased oleic-acid concentration (Ol and Ol2) and three loci for reduced linolenic-acid concentration (Ln, Ln1, Ln2) in the Ethiopian mustard mutants N2-3591, with increased oleic-acid concentration, and N2-4961 and HF-186, both having reduced linolenic-acid concentration. Novel Ethiopian mustard oil types with several increased levels of oleic acid combined with reduced linolenic-acid concentration, both in zero erucic- and high erucic-acid backgrounds, were developed after genetic recombinations between the mutants followed by transfer of the novel traits to the zero erucic-acid line 25X-1. The highest oleic-acid concentration (83.9%) was achieved in the line AB01323, which also exhibited a reduced linolenic-acid concentration (5.0%). This line is hypothesized to have the genotype *ololol2ol2LnLnln1ln1ln2ln2*. An additional reduction of linolenic-acid and a subsequent increase of oleic-acid concentration in AB01323 is expected by introgressing the *ln* alleles from the N2-4961 mutant.

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