Novel seed oil types in Ethiopian mustard

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ABSTRACT

Current zero erucic acid germplasm of Ethiopian mustard is characterized by high levels of polyunsaturated fatty acids (>35% linoleic acid; >20% linolenic acid) and low levels of monounsaturated oleic acid (<35%). Several mutants and breeding lines with altered levels of these fatty acids, including high oleic acid and low linolenic acid types, have been developed in high-erucic acid Ethiopian mustard. The objective of the present research was to develop novel seed oil types of Ethiopian mustard through recombination among these lines followed by transfer to zero erucic acid material. Transgressive recombinants produced in crosses between different lines with altered fatty acid profile were crossed with zero-erucic acid plants. F_2 populations were nondestructively screened at a single-seed level by near-infrared reflectance spectroscopy (NIRS) followed by confirmatory half-seed analyses by gas-liquid chromatography. After selection in the F_2 and F_3 generations, zero erucic acid recombinants with novel seed oil types were confirmed in the $F_{3:4}$ generation. The recombinants included mid oleic (>65%), high oleic (>75%), high linoleic (>55%), low linolenic (<7%), very low linolenic (<3%), and high linolenic acid (>30%) oil types, together with combinations of some of them, as for example high oleic/low linolenic acid (>82% oleic, <7% linolenic acid). Preliminary information on the inheritance of some of the novel traits is reported.

Key words: Brassica carinata – high oleic acid – high linoleic acid – high linolenic acid - low linolenic acid

INTRODUCTION

The oil quality of *Brassica* species is mainly characterized by its fatty acid composition, which can be modified according to a wide range of nutritional and industrial end uses. Zero erucic acid forms of Ethiopian mustard (*Brassica carinata* A. Braun) are characterized by a seed oil exceptionally rich in polyunsaturated fatty acids (linoleic and linolenic acid). Thus, zero erucic acid Ethiopian mustard oil typically contains 33% oleic acid (18:1), 37% linoleic acid (18:2), and 21% linolenic acid (18:3) (Alonso et al. 1991; Getinet et al. 1994; Fernández-Martínez et al. 2001), compared to 61% oleic acid, 21% linoleic acid, and 11% linolenic acid in canola (Scarth and McVetty 1999). As polyunsaturated fatty acids are very susceptible to oxidative processes that deteriorate the quality of the oil, their partial substitution by monounsaturated oleic acid is an important breeding objective for producing Ethiopian mustard oil with high oxidative stability. But on the other hand, oils rich in polyunsaturated fatty acids are of great value for both nutritional and industrial applications. Both linoleic (omega-6) and linolenic acid (omega-3) are essential fatty acids with beneficial effects on human health (Harper and Jacobson, 2001). Also, oils rich in polyunsaturated fatty acids possess a number of industrial applications, especially in the industry of paints, varnishes, inks and coatings (Büchsenschütz-Nothdurft et al., 1998).

Several mutants and breeding lines of Ethiopian mustard having altered levels of oleic, linoleic, and linolenic acid have been developed in high erucic acid material (Velasco et al., 1997a,b). The objective of the present research was to develop novel seed oil types of Ethiopian mustard through genetic recombination among these lines followed by transfer to zero erucic acid background.

MATERIALS AND METHODS

The present research was conducted with the following Ethiopian mustard lines: N2-3591, with increased oleic acid, and N2-4961, with reduced linolenic acid, both with standard high erucic acid concentration and developed by mutagenesis (Velasco et al., 1997a); HF-186, with

reduced linolenic acid and standard high erucic acid concentration, developed by pedigree selection (Velasco et al., 1997b); 25X-1, with zero-erucic acid concentration and standard high levels of polyunsaturated fatty acids, developed by interspecific crossing (Fernández-Martínez et al., 2001). The fatty acid profile of the three lines, together with the line C-101 of standard fatty acid profile, is shown in Table 1.

Table 1. Mean and standard deviation of major fatty acids (% 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.

Line	16:0[a]	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]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.

The high erucic acid lines N2-3591, N2-4961, and HF-186 were crossed following a diallel design. Transgressive F_2 recombinants were carried to F_3 and crossed with the zero-erucic acid line 25X-1. Screening for zero erucic acid concentration in segregating F_2 seeds was nondestructively conducted at the single-seed level using near-infrared reflectance spectroscopy (NIRS), and putative zero-erucic acid seeds were further analysed for fatty acid profile by gas-liquid chromatography (Velasco et al., 2003). As zero-erucic acid F_2 seeds widely segregated for oleic, linoleic, and linolenic acid levels, a selection for different levels of these fatty acids was conducted in the F_2 and F_3 generations. The results presented in this paper include information of the $F_{3:4}$ generation.

RESULTS

High oleic/low linolenic acid recombinants in high erucic acid background with similar levels to the parents (24% oleic acid, 6% linoleic acid) were obtained from crosses between the high oleic acid line N2-3591 and the low linolenic acid line N2-4961. However, crosses between N2-3591 and the low linolenic acid line HF-186 produced transgressive segregants with a higher oleic acid content than the high oleic acid parent (up to 29.7% oleic acid, compared to a maximum of 25.1% in N2-3591). Similarly, transgressive segregants, with linolenic acid concentration of 1.8%, were obtained from crosses between the low linolenic acid lines N2-4961 and HF-186, both with linolenic acid content above 3.5%. Both types of transgressive segregants in high erucic acid background were uniformly expressed in the F_3 (Fig. 1).

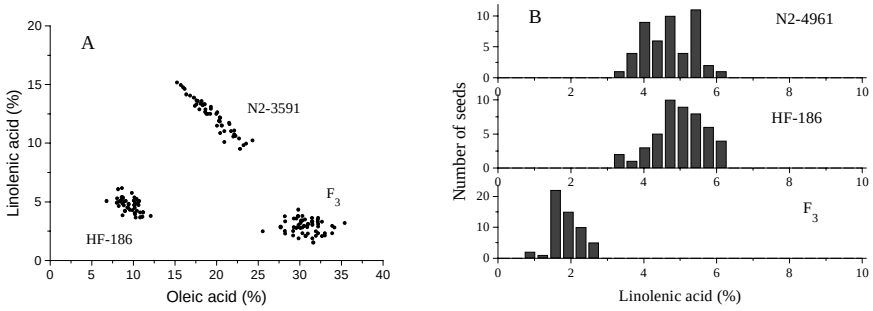

Fig. 1. Oleic and/or linolenic acid concentration in transgressive F_3 and parental seeds from crosses between the high oleic acid line N2-3591 and the low linolenic acid line HF-186 (A), and from crosses between the low linolenic acid lines N2-4961 and HF-186 (B).

Transgressive F_3 plants were crossed with the zero-erucic acid line 25X-1. After initial screening for erucic acid content, zero erucic acid F_2 seeds were found to show a large variation for oleic, linoleic and linolenic acid contents. Zero erucic acid F_2 seeds ranged from 16.6 to 87.6% oleic acid, 2.5 to 42.1% linoleic acid, and 4.1 to 21.1% linolenic acid in the cross N2-3591/HF-186//25X-1, and from 29.0 to 44.8% oleic acid, 37.8 to 55.1% linoleic acid, and 2.5 to 16.7% in

the cross N2-4961/HF-186//25X-1. Selection in the F_2 and F_3 produced a set of $F_{3:4}$ lines with contrasting fatty acid profiles (Table 2). Preliminary genetic characterization of this material revealed the presence of two genes for high oleic acid and three genes for low linolenic acid content. Expected allelic configurations for the lines are presented in Table 2.

Table 2. Mean and standard deviation of C18 major fatty acids (% of the total fatty acids) of the zero erucic acid Ethiopian mustard line 25X-1, with standard fatty acid composition, seven $F_{3:4}$ lines with different fatty acid profiles, and expected allelic configurations.

Line	Type[a]	18:1[b]	18:2	18:3	Expected allelic configuration
25X-1	Standard	32.9 ± 3.5	42.6 ± 2.3	16.4 ± 2.4	*OlOlOl2Ol2LnLn Ln1Ln1Ln2Ln2*
AB01169	MO	70.7 ± 2.4	15.0 ± 1.8	7.5 ± 1.5	*ololOl2Ol2LnLnln1ln1Ln2Ln2*
AB01066	HO	79.5 ± 1.4	4.8 ± 0.8	8.7 ± 1.3	*ololol2ol2LnLn ln1ln1Ln2Ln2*
AB01323	HO/LLn	83.9 ± 1.0	4.2 ± 0.4	5.0 ± 0.7	*ololol2ol2LnLn ln1ln1ln2ln2*
AB01345	LLn	39.4 ± 1.9	48.1 ± 3.1	5.8 ± 0.6	*OlOlOl2Ol2Ln Lnln1ln1ln2ln2*
AB01356	VLLn	40.7 ± 1.4	50.2 ± 1.1	1.7 ± 0.4	*OlOlOl2Ol2lnln ln1ln1ln2ln2*
AB02045	HL/VLLn	30.8 ± 3.2	60.6 ± 3.4	1.4 ± 0.6	*Not determined*
AB02105	HLn	19.4 ± 2.7	34.5 ± 4.5	38.4 ± 4.8	*Not determined*

[a]MO=mid oleic, HO=high oleic, LLn=low linolenic, VLLn=very low linolenic, HL=high linoleic, HLn=high linolenic
[b]18:1=oleic acid, 18:2=linoleic acid, 18:3=linolenic acid

DISCUSSION

The present research has produced zero-erucic Ethiopian mustard germplasm with new seed oil types, some of them similar to currently existing canola types such as mid oleic, high oleic, or low linolenic (Scarth et al., 1999), but others having novel fatty profiles of great value for nutritional and industrial applications (e.g. high linoleic and high linolenic acid types). The availability of such a diversity of fatty acid profiles, together with the optimal agronomic adaptation of the crop to the Mediterranean environment, should encourage greater efforts for the development of Ethiopian mustard as an important oilseed crop for Mediterranean agriculture.

REFERENCES

Alonso, L.C., O. Fernández-Serrano, and J. Fernández-Escobar, 1991: The outset of a new oilseed crop: *Brassica carinata* with low erucic acid. In: Proc 8th Int Rapeseed Congr, Saskatoon, Canada. GCIRC, pp 170-176. GCIRC, Paris.

Büchsenschütz-Nothdurft, A., A. Schuster, and W. Friedt, 1998: Breeding for modified fatty acid composition via experimental mutagenesis in *Camelina sativa* (L.) Crtz. Ind. Crops Prod. 7, 291-295.

Fernández-Martínez, J.M., M. Del Río, L. Velasco, J. Dominguez, and A. De Haro, 2001: Registration of zero erucic acid Ethiopian mustard genetic stock 25X-1. Crop Sci. 41, 282.

Getinet, A, G. Rakow, J.P. Raney, and R.K. Downey, 1994: Development of zero erucic acid Ethiopian mustard through an interspecific cross with zero erucic acid Oriental mustard. Can. J. Plant Sci. 74, 793-795.

Harper, C.R. and T.A. Jacobson, 2001: The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. Arch. Int. Med. 161, 2185-2192.

Scarth, R. and P.B.E. McVetty, 1999: Designer oil canola. A review of food-grade Brassica oils with focus on high oleic, low linolenic types. Proc. 10th Int. Rapeseed Congr., Canberra, Australia, CD Rom.

Velasco, L., J.M. Fernández-Martínez, and A. De Haro, 1997a: Induced variability for C18 unsaturated fatty acids in Ethiopian mustard. Can. J. Plant Sci. 77, 91-95.

Velasco, L., J.M. Fernández-Martínez, and A. De Haro, 1997b: Selection for reduced linolenic acid content in Ethiopian mustard. Plant Breeding 116, 396-397

Velasco, L., A. Nabloussi, A. De Haro, and J.M. Fernández-Martínez, 2003: Development of high oleic, low linolenic acid Ethiopian mustard (*Brassica carinata*) germplasm. Theor. Appl. Genet. (in press).