THE OUTSET OF A NEW OILSEED CROP: BRASSICA CARINATA WITH LOW ERUCIC ACID CONTENT

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INTRODUCTION

Abyssinian mustard (*Brassica carinata* Braun), an allotetraploid species derived from the diploid species *B. nigra* (L.) Kosch, and *B. oleracea*. (U, 1935), is grown in the Indian subcontinent and Ethiopia for the production of vegetable oil. *B. carinata* has certain advantages over its closely related species, rape (*B. napus* L.) and turnip rape (*B. campestris* L.) including a lower susceptibility to shattering; greater yield potential in countries with a dry climate (Knowles *et al*, 1981; Fernández-Martínez and Domínguez, 1982; Ferreres *et al.*, 1983; Prakash *et al.*, 1984) as well as greater resistance to most of the diseases and pests which commonly attack rape and turnip rape (notably blackleg) (Riley and Belayned, 1982; Prakash *et al.*, 1984; Sacristan and Gerdeman, 1986; Anand *et al.*, 1985; Sing *et al.*, 1988).

Despite the agronomical interest of *B. carinata* there has been very little effort to improve it as an oil crop either for industrial use or else for nutritional purposes. However the identification of plants with essentially no erucic acid in their seed oil in *B. napus* (Stefansson *et al.*, 1961) and *B. campestris* (Downey, 1964), resulted in the world-wide development of nutritionally superior low erucic oil bearing cultivars. The isolation of zero erucic acid in *B. juncea* plants (Kirk and Oram, 1981; Anand and Robbelen, 1984) is the basis for the development of this species as an edible oil crop. Two *loci*, each with several alleles, determine the level of erucic acid in the seed oil of *B. napus* (Harvey and Downey, 1964) as well as in the seed of *B. juncea* (Kirk and Hurlstone, 1983). In *B. campestris* two alleles at a single locus control erucic acid synthesis (Dorrel and Downey, 1964).

Different *Cruciferae* species have been studied as potential sources of high erucic acid oil. (Appelqvist and Jönsson, 1970). The highest amounts, somewhat above 60%, were found in summer turnip rape, winter rape and crambe (*Crambe abyssinica* L.). Additional selection efforts in these species did not yield any positive effect.

In 1981, a conventional breeding programme was started at Koipesol, S.A. for the isolation of *B. casrinata* inbred lines with superior agronomical characters and for the improvement of the seed oil quality, both either by reducing or increasing the erucic acid content for nutritional or industrial uses from its natural range (35-40%), respectively.

MATERIAL AND METHODS

The *B. carinata* seed samples used as starting material were mainly from the collection available at the United States Department of Agriculture. There were 15 ecotypes from Ethiopia sent to Spain by Dr. P.F. Knowles in 1981 and kindly suplied to us by Dr. Fernández-Martinez from the INIA at Córdoba. 56 new ecotypes of this species were kindly sent to us by D.K. Downey from the Canadian Department of Agriculture in 1983.

The breeding methodology was based on a pedigree breeding system. Selfed plants were selected during the first 2 years in order to develop homogeneous lines. F3 lines were classified by their erucic acid content and selfed plants were selected in the most promising lines. After the classification of these single plants by their erucic acid content, only those plants having either reduced or increased the erucic acid content were planted in the F4 nursery. Crosses among plants of the most promising lines were performed at F3, F4 and F5 generations. These crosses were made between plants with either the same or a different genetic origin.

Different quantities of seed were used for the chemical analyses, depending on the amount available and the nature of the sample. The analysis of original populations and inbred lines were generally performed with 5-10 g. samples. Selfed single plants were analysed with 1 g. samples. Twenty single seed analyses were performed in those selfed plants with an erucic acid content either higher or lower than the average. These analyses were performed in order to find out if a genetic segregation was present in the promising plants. From the erucic acid pattern of the individual seeds, the selfed plants were selected. A large scale screening utilising half seed technique (Downey and Harvey, 1963) was carried out on the remaining seed of these plants.

A total of 2.060 plants and lines were analysed for fatty acid composition in this breeding program. The half seed technique analysis was made on 693 seeds. Fatty acid compositions were determined by gas chromatography of methyl esters prepared by standard procedures. Most of these analyses were performed at the main laboratory of the Spanish edible oil firm Koipe, S.A. In the last year of this work, the large scale screening utilising the half seed technique was performed both at the Koipe, S.A. laboratory and in Sweden, at the laboratory of the seed firm Svalof AB.

Exponential or polimomial regression lines were calculated between the content of erucic acid and the different fatty acids of the seed oil of B. carinata. These regressions lines, were plotted using the Harvard Grafic 2:10 program from the Sofware publishing Corporation.

RESULTS AND DISCUSSION

The breeding efforts during the 1981/82 and 1982/83 seasons were concentrated on the isolation of homogenous B. carinata lines. Selection for either increased or reduced erucic acid content started in 1984. Table 1 summarizes the erucic acid contents found in the Koipesol B. carinata nurseries between 1983 and 1990.

A variation in erucic acid content of between 27% and 55% was obtained from 153 B. carinata lines analysed in 1984.

1) Low Content of Erucic Acid

In 1984 only 5 B. carinata lines were found with erucic acid content below 34%. Selection during 1984/85 among selfed plants from these lines allowed the identification of 10 plants with erucic acid content below 34%. During the 1985/86 season eight lines were found with erucic acid content between 19% and 27%. Since several of these lines breed true for the erucic acid content, artificial crosses were made between individual plants from these lines. Among different F2 segregating plants of these crosses, only one plant was found with erucic acid content of 10,8 (Fernández-Serrano and Alonso, 1988). Unfortunatly these plants were lost, and another one was found in 1988 with 13,5 of erucic acid.

If the inheritance of erucic acid content in B. carinata follows a similar pattern to the one found in B. napus (Harvey and Downey, 1964) and in B. juncea (Kirk and Hurlstone, 1983), two loci with several alleles are expected to determine the erucic acid content in the Abyssinian mustard (Fernández-Escobar et al., 1988). At least two kinds of allele, Eo and E_1 contributing 0%-2% and 12%-15% erucic acid, respectively, are expected for each of the two loci. Thus, most of the lines that breed true for erucic acid content between 19% and 27% would have been either E_1E_1-EoEo or else EoEo-E_1E_1. Since many crosses among them did not yield any further reduction in the erucic acid content, most of them would have had the same locus with the Eo homozygous allele. Only the plant with 13.5% of erucic acid was supected to be either EoEo-EıEo or EıEo-EoEo. Screening by the half seed technique among the offspring seed of this plant allowed the isolation of 22 seeds between 0% and 2% and with 29% being the highest erucic acid content found. These results support the above mentioned hypothesis about the inheritance of erucic acid content in B. carinata, although probably more than two alleles are expected to be operating for each locus as happens in other Brassica species. Twenty plants fully developed to maturity producing seeds with oil essentially free of long chain fatty acids (Table 2).

2) High Content of Erucic Acid

Crosses among B. carinata plants with more than 50% of erucic acid content in their seed oil were made in 1987. Most of the segregating F2 plant had erucic acid content between 45-55% and with the highest values near 55%. These results indicate that breeding for high erucic acid is not easy above 55%. On the other hand the it was possible to isolate plants with an erucic acid content between 60 and 61,4% by the pedigree breeding system.

In Brassica seed oil, it seems that erucic acid can only be attached to positions 1 and 3 of the glycerol (Appelqvist 1971), from which it follows that 66-67% erucic acid is the maximum attainable level. Given competition from other fatty acids the plant would probably have difficulty in achieving this high values. The values attained in B. carinata are among the highest found in the Brassica and Crambe species (Appelqvist and Jönsson, 1970; Kumar and Tsunoda, 1980).

3) Changes of the B. carinata Seed Oil as its Erucic Acid Content is Altered

The changes in fatty acid composition in the Brassica seed oils caused by either an increase or a reduction of the erucic acid content has been used to study the relationship between the phylogeny and differentiation of fatty acid biosynthesis (Tsunoda and Kumar, 1976). From the nutritional stand point, a higher concentration of monoenoic (oleic acid, C18:1) and dienoic (linoleic acid, C18:2) fatty acids are favorable, while trienoic (linolenic, C18:3) fatty acid is unfavorable because its three double bonds are sensitive to oxidation, thus leading to bad taste and flavor during the heating and storage. It is therefore, desirable to decrease as much as possible the linolenic acid content from the low erucic cultivars of the Brassica species.

Whereas the linoleic acid content does not change much when the erucic acid content is reduced in B. napus, B. campestris and B. juncea (Table 2), in the B. carinata, the reduction of erucic acid content causes an increase of the three 18 carbon fatty acids, C18:1, C18:2 and C18:3. Linolenic acid increases almost to double the content found in the standard B. carinata lines. These results suggest that the enzymes or system of enzymes which seem to be operating in the synthesis of linolenic acid in B. carinata, are more effective than those operating in B. napus, B. juncea and B. campestris.

In order to find out the correlation between the different fatty acid and erucic acid content, 82 chromatographs were selected with erucic acid between 0 and 60%. Figure 1 illustrates the different exponential and parabolic regression lines for the different fatty acids as erucic acid increases from 0 to 60%. Palmitic acid had a lineal regression line, whereas eicosenoic acid had a parabolic regression line similar to the results found in B. napus (Jönsson, 1977). The three 18 carbon fatty acids, had exponential regression lines but with different slopes, being oleic acid the one with the most pronounced slope.

The individual values of linolenic acid content have also been plotted in fig. 1. There are a few plants within the standard B. carinata types (35 to 50% erucic acid) which had between 7% and 8% of linoleic acid. These results indicate that it would be possible to develop low erucic B carinata with linolenic acid content values similar to those found in other low erucic Brassica species: i.e., from 7% to 12%. On the other hand, the existence within the standard erucic acid content B. carinata lines, of plants with linolenic acid content above 18%, suggests the possibility of increasing the content of this fatty acid above 30% in a low erucic background. An increase of linolenic acid above 50%, would allow the use of B. carinata oil as a substitute of flax oil. Present breeding efforts in our program focus now on both the reduction and the increase of linolenic acid in low erucic B. carinata.

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Table 1: Erucic acid content in the B. carinata breeding program of Koipesol between 1983 and 1990

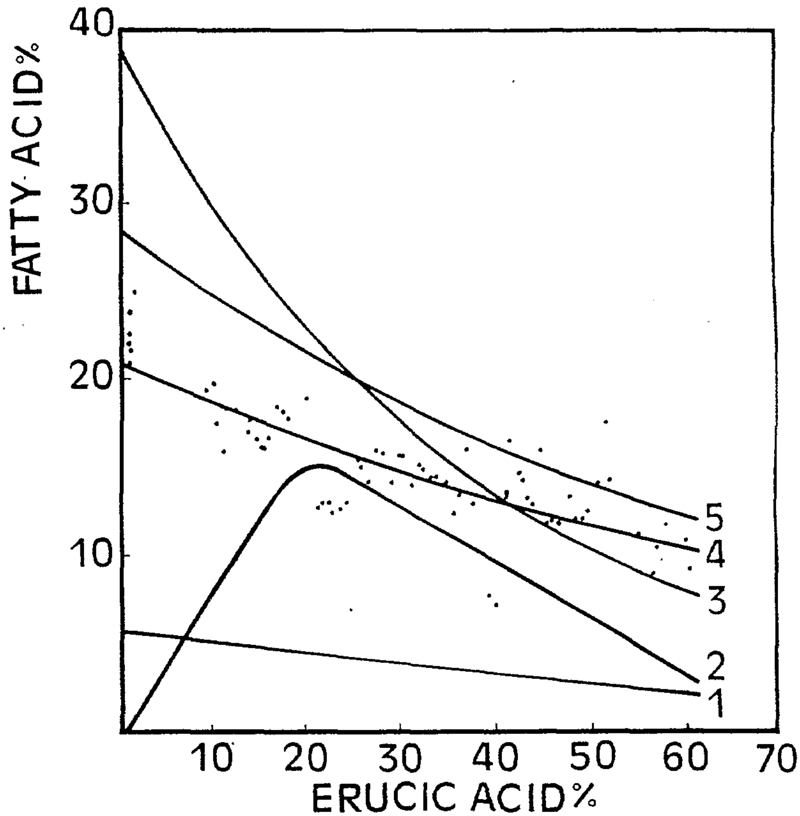
YEAR	ERUCIC ACID CONTENT						
	<2%	2-17%	17-27%	27-34%	34-45%	45-55%	>55%
83/84	0	0	0	5	140	8	0
84/85	0	0	0	6 (10)	150 (48)	9 (5)	0
85/86	0	0	8 (14)	56 (26)	123	10 (8)	0
86/87	0	0	21 (39)	40	103	10 (11)	0
87/88	0	0	47(244)	35(136)	110	11 (40)	0
88/89	0 (22)	0 (48)	45	42(200)	79	8 (94)	0 (11)
89/90	20	-	-	-	-	-	5

Numbers between brackets indicate individual selfed plants
Numbers without brackets indicate bulked seed from lines

Table 2: Fatty acid composition (%) of the Brassica species in which cero erucic mutants has been found

SPECIE	PALMITIC 16:0	ESTEARIC 18:0	OLEIC 18:1	LINOLEIC 18:2	LINOLENIC 18:3	EICOSENOIC 20:1	ERUCIC 22:1
B. napus							
Standard	3,00	0,80	9,90	13,50	9,80	6,80	53,60
Low 22:1	4,90	1,40	56,40	24,20	10,50	1,20	0,0
B. campestris							
Standard	1,80	0,90	13,10	12,00	8,20	6,20	55,50
Low 22:1	3,80	1,20	58,60	24,00	10,30	1,00	0,30
B. juncea							
Standard	2,50	1,20	8,00	16,40	11,40	6,40	46,20
Low 22:1	3,60	2,00	45,00	33,90	11,80	1,50	0,10
B. carinata							
High 22:1	3,00	0,90	7,10	11,60	10,80	4,70	60,00
Standard	3,19	1,05	13,75	17,36	12,63	10,52	40,10
Midle 22:1	3,60	1,00	25,64	15,10	12,70	16,10	23,90
Low 22:1	5,75	1,20	39,00	29,50	22,20	1,10	0,18

Fig. 1: Exponential and parabolic regression lines for the different fatty acids as erucic acid increases from 0% to 60% in *B. carinata* genotypes



5 Linoleic acid
4 Linolenic acid
3 Oleic acid
2 Eicosenoic acid
1 Palmitic acid

Dots represents observed values for this fatty acid.