QUALITY STUDIES IN INTERSPECIFIC HYBRIDS OF BRASSICA

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Ethiopian mustard (Brassica carinata) is grown as an oil seed crop in Ethiopia. Recently there has been a great interest on this crop in some countries and particularly in Spain, where it has been tested as an alternative to rapeseed (B. napus). Its potentiality as an oil crop has been evaluated for several years in southern Spain, showing a higher yield potential and vigor than B. napus and B. juncea (Fernández-Martínez and Domínguez, 1982; Fereres et al, 1983). Similar results have been obtained in California (Knowles et al., 1981) and in India, where besides showing a high yield potential, this species has been found to be quite resistant to stress conditions as well as to some diseases (Prakash et al., 1984).

Unfortunately, available material of this species lacks of two seed quality traits necessary to be used as an edible oil and protein crop: erucic acid-free oil and glucosinolate-free meal. Both traits are present in B. napus and B. campestris and double zero varieties have been developped in Canada and Europe. Zero erucic B. juncea has also been obtained (Kirk and Oram, 1981). In spite of the increasing interest in B. carinata, as far as we know, there is no information of the existence of variability in this specie for these two characteristics.

B. carinata is an allotetraploid species (2n=34:BBCC) originally coming from a cross between B. nigra (2n=16:BB) and B. oleracea (2n=18:CC) (U,1935). Metaphase I analyses of hybrids between B. napus and B. carinata and between B. juncea and B. carinata show a complete pairing between the chromosomes corresponding to the common genomes, C in the first cross and B in the second respectively (Harper and McCarthur, 1980).

Therefore, it is possible by interspecific hybridization between B. carinata and the other available species with low levels of one or both quality traits to obtain genetic introgression from these last species into B. carinata, with the ultimate goal of obtaining double zero mustard varieties. Such a program has been initiated in our Centre. Preliminary data concerning content of

erucic acid in the first developed hybrids are presented in this paper.

MATERIALS AND METHODS

Parental material used in this program has been:
B. napus sp. oleifera, Cv. DUPLO (low erucic and low glucosinolate: "double zero").
B. juncea Cv. Zem-1 (low erucic acid) received from Dr. A. Kirk, CSIRO-Australia.
B. carinata. Selection from an original population provided by Dr. P.F. Knowles (University of California, Davis), which we have identified as C-101.
Rapid cycling B. oleracea and B. nigra, provided by Dr. P. Williams, University of Wisconsin.

Crosses were made in a greenhouse with a temperature setting of 20-25ºC and 12 hours of daylight.

Determination of the true nature of the hybrid seed was done after somatic chromosome counting on root tips. These were pretreated by washing in tap water at 0ºC during 22 hours in a Cl_2Ca solution 5 M fixed in acetic acid: absolute ethanol (1:3) and stained by the Feulgen procedure.

Fatty acid analyses of generations F_1, F_2 and BC_1F_1 were carried out on oil extracted from individual half-seeds. The remaining halfs were grown on orchid agar in controlled growth chambers. Parental seed analyses were performed on complete single seeds.

Seed oil was extracted using re-distilled petroleum ether. Methyl esters of the fatty acids were used for gas chromatographic analyses. Formation of methyl esters was accomplished by adding 1 ml of Sodium methylate to the oil and refluxing for five minutes using air condensers. After cooling, sulfuric methanol was incorporated until neutralization. Then 0.25 ml of hexane were added and floating methyl esters were injected into the gas chromatograph. This was a Hewllet-Packard, model 5830-A using a flame ionization detector and electronic integrator. The stainless column 2 m in length and 3.2 mm in diameter was packed with 5% diethylene glycol succinate on 80/100 W/AW Chromosorb. Column temperature 190ºC, injector temperature 285ºC and detector temperature 250ºC. The Nitrogen carrier gas meter reading was 20 ml/min.

RESULTS AND DISCUSSION

The following interspecific hybrids were obtained:
a) Duplo X C-101 and reciprocal.
b) Zem-1 X C-101 and reciprocal.
c) Zem-1 X B. nigra.
d) Duplo X B. oleracea.

The production of these interspecific hybrids corresponds to two different strategies for transferring low erucic gene(s) from B. napus and B. juncea to B. carinata. In crosses a) and b) it is intented the introgression from genomes C from B. napus and B from B. juncea into the same genotypes of B. carinata and eventually allosyndetic pairing between the different genomes. In crosses c) and d), the aim is to obtainin a low erucic B. nigra and B. oleracea and a later synthesis of B. carinata by hybridization and chromosome duplication. Fatty acid analyses of F_1 seed were carried out in half seeds. The remaining halves were germinated and the somatic chromosome number was determined. A few anomalous numbers of chromosomes, as for example 55 in seeds of cross a), and some selfings appeared along with F_1 seeds which showed 36, 35, 26 and 28 chromosomes in crosses a), b), c) and d) respectively.

Fatty acid analyses of the F_1 seeds from the hybrid between tetraploid species (Duplo X C-101, Zem-1 X C-101 and reciprocals) show erucic acid contents close to the mean of the parental (Fig 1 and Fig 2). These results suggest that no dominant effects are present among the loci (locus) coming from the different species. Additive action seems to act as was previously found in B. napus (Harvey and Downey, 1964) and in B. juncea (Kirk and Hursltone, 1983). There were some differences in erucic acid content between the cross Zem-1 X C-101 and its reciprocal which averaged 26.33 % and 34.38 % respectively (Fig. 1). Although it could suggest some cytoplasmic effect on the control of erucic acid content, the number of analysed seeds in the cross C-101 X Zem-1 hybrid was low and further analyses should be carried out to confirm this hypothesis. On the other hand environmental differences during seed development can be important for erucic acid (Harvey and Downey, 1964) and could also explain the observed differences.

Only one triploid hybrid (cross between tetraploid and diploid species) Zem-1 X B. nigra was analysed. The hybrid seeds from the cross between Duplo and B. oleracea were not analysed due to an abnormal development of the F_1 seeds. The half seed technique might likely have damaged the embryo in these seeds.

Zem-1 X B. nigra F_1 seeds showed an erucic acid content of 32.1 % (Fig. 1). This value is 10 points above of the mean content of parental species (21.62 %), suggesting a partial dominant effect of high erucic. A similar effect has been described in crosses involving B. campestris varieties from Bangladesh (Moller et al., 1985).

Results of the backcross to B. carinata ((Duplo X C-101) X C-101) are shown in Fig. 2. It can be noticed that mean erucic acid contents of BC_1F_1 are located in between parental mean and high values. Three classes can be made, with mean erucic values of 20.0 %, 33.9 % and

47.7 % respectively. These groups can be explained according to the genetic system already set up in B. napus (Harvey and Downey, 1964; Morice, 1974); erucic acid being controlled by two genes displaying no dominance and acting in an additive manner, and each individual gene located in a different genome (Anand and Downey, 1981). The groups above mentioned might correspond to the following genetic constitution: $E_1e_1E_2e_2$ for the group of 20 %, $E_1E_1E_2e_2$ or $E_1e_1E_2E_2$ for the 34 % group and $E_1E_1E_2E_2$ for the 47.75 % one.

The obtained results shown in Table 1 seem to confirm this model which presents the best fit when compared with different hypothesis of erucic acid in B. carinata. Expected results in Table 1 are based on a genome constitution CCAB and a limited recombination among genomes A and B (Rousselle and Eber, 1983). A 0.4 frequency was calculated for the allosyndetic pairing between A and B genomes and/or the loss of the chromosome carrying the gene controlling erucic acid in genome B.

F_2 data (Fig 2) support this hypothesis. Erucic acid content of individual F_2 seeds varied from zero to high values (similar to the ones of B. carinata). Due to the relatively low number of seeds analysed, no attempt was made to group the phenotypes into classes. However, low erucic acid contents in F_2 (0 % and 10-20 %) as well as the 20 % group in BC_1F_1 are probably due to chromosome losses and allosyndetic pairing as discussed previously.

Although further analyses and crosses are needed to confirm the presented results, the data obtained in this work show the possibility of obtaining B. carinata germplasm with low values of erucic acid. Therefore, broad programs of interspecific hybridization in Brassica may be of great interest in creating variability for seed quality traits in some species of this genus.

TABLE 1.- POSTULATED GENOTYPES, EXPECTED AND OBSERVED FREQUENCIES AND ERUCIC ACID CONTENT IN THE BC_1F_1 GENERATION OF THE CROSS (DUPLO X C-101) X C-101

BC_1 GENOTYPES	FREQUENCY		ERUCIC ACID %	
	EXPECTED	OBSERVED	RANGE	MEAN
$E_1e_1E_2e_2$	9.0	7	17.2-21.2	20.0
$E_1E_1E_2e_2/E_1e_1E_2E_2$	22.5	25	28.5-41.9	33.9
$E_1E_1E_2E_2$	13.5	13	43.9-52.7	47.7

$X^2 = 0.66$ $\quad 0.70 < P < 0.80$

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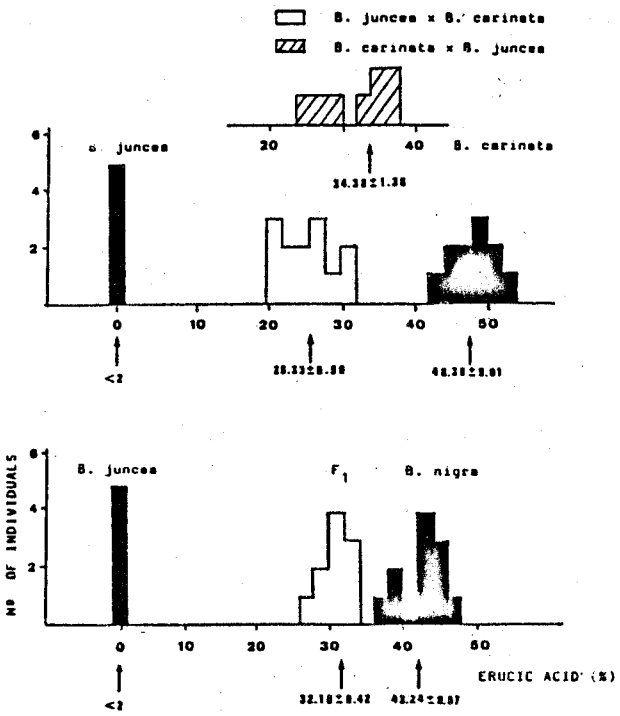

Fig. 1. Erucic acid % distribution of F_1's from crosses between B. juncea x B. carinata and reciprocal and B. juncea x B. nigra.

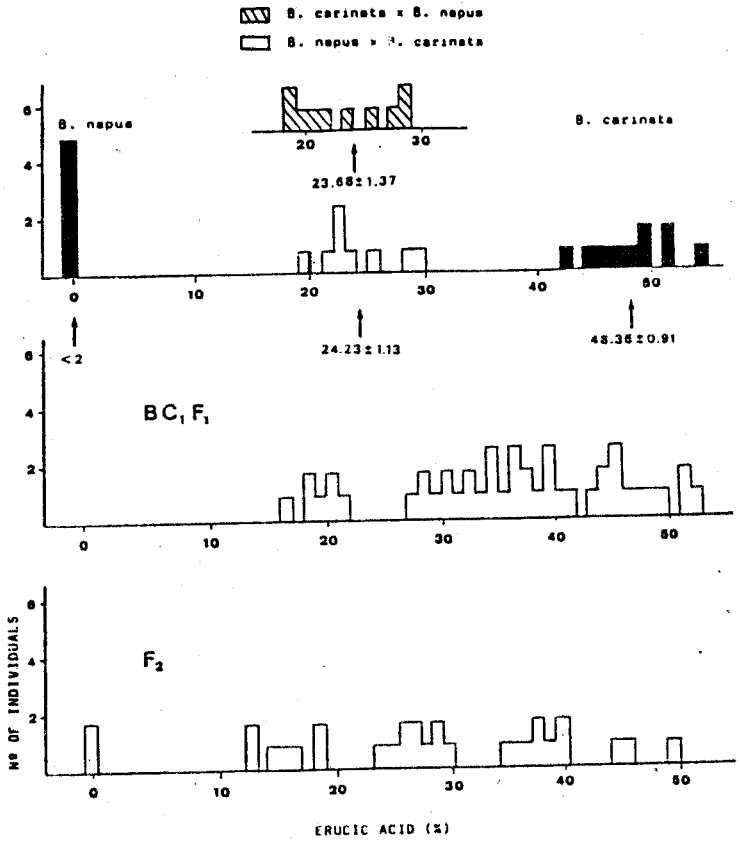

Fig. 2. Erucic acid % distribution of: F_1 from the cross B. napus x B. carinata and reciprocal, BC_1F_1 (B. napus x B. carinata) x B. carinata and F_2 B. napus x B. carinata.

REFERENCES

ANAND, I.J and R.K. DOWNEY. 1981. A study of erucic acid alleles in digenomic rapessed (Brassica napus L.). Can J. Plant Sci. 61: 199-203.

FERERES, E., J. FERNANDEZ, I. MINGUEZ and J. DOMINGUEZ. 1983. Productivity of Brassica juncea and B. carinata in relation to rapessed. In Proceedings 6th. Congrés International sur le colza. Paris. France 293-293.

FERNANDEZ-MARTINEZ, J. and J. DOMINGUEZ. 1982. Aspects and progress of oil crop breeding in Spain. In "Production and utilization of protein oil seed crops" Ed. I.S Bunting. World Crops. Vol. 5 Marthinus Nijhoff. The Hague. Netherland. pp:50-57.

HARBERD, D.J. and E.D. McARTHUR. 1980. Meiotic analyses of some species and genus hybrids in the Brassiceae. In "Brassica crops and wild allies". Ed. Tsunoda, Hinata and Gómez-Campo. pp:65-88.

HARVEY, B.L. and R.K. DOWNEY. 1964. The inheritance of erucic acid content in rapeseed (Brassica napus L.). Can J. Plant Sci. 44:104-111.

KIRK, J.T.O. and R.N. ORAM. 1981. Isolation of erucic-free lines of Brassica juncea: Indian mustard now a potential oil seed crop in Australia. J. Aust. Inst. of Ag. Sci. 61:51-52.

---------- and C.J. HURLSTONE. 1983. Variation and inheritance of erucic acid content in Brassica juncea. Z. Pflanzenuchtg. 90:331-338.

KNOWLES, P.F., T.E. KEARNEY and O.B. COHEN. 1981. Species of rapeseed and mustard as oil crops in California. Chapter 18 in "New sources of fats and oils". Ed. E.H. Pryde, L.H Prince and K.D. Mukherjie. American oil chemist society, Champain. Illinois.

MOLLER, P., M.H. RAHMAN, O. STOLEN and H. SORENSEN. 1985. Heredity of fatty acids and glucosinolates in oil seed rape. Possibilities for improvement of rape adapted for the growth conditions in tropical Asia. In "Advances in the production an utilization of the cruciferous crops. Ed. H. Sorensen. pp.2286-300.

MORICE, J. 1974. Selection d'une variété de colza sans acide érucique et sans glucosinolates. In Proceedings of 4th. International rapeseed conference. Giessen. F.D.R. 31-47.

PRAKASH, S., S. GUPTA, R.N. RAUT and A.K. KALRA. 1984. Synthetic Brassica carinata - a preliminary report. Cruciferae news. 9:36.

ROUSSELLE, P. and F. EBER. 1983. Croisement interspecifiques entre quelques Brassiceae et Brassica napus L. Analyse génomique des hybrides et perspectives d'obtention de systémes dándroestétilité chez la colza. Agronomie 3:153-159.

U, N. 193. Genome Analysis in Brassica with special reference to the experimental formation of B. napus and peculiar mode of fertilization. Jap. J. Bot. 7:389-392.