MORPHOLOGICAL MUTANTS IN ETHIOPIAN MUSTARD. ISOZYME AND BIOCHEMICAL CHARACTERIZATION

A. DE HARO, L. VELASCO, J. FERNANDEZ-MARTINEZ

Instituto de Agricultura Sostenible. C.S.I.C. Apartado 4084. 14080 Córdoba, Spain

M. DEL RIO

Centro de Investigación y Desarrollo Agrario. Apartado 4240. 14080 Córdoba. Spain

ABSTRACT

Three types of morphological mutants were identified in M2 generation of Etyl Metane Sulfonate treated seeds: apetalous mutant (APM) with reduced or no petals, speck sepals mutant (SSM) with speckled sepals and opened pods, and curl petals mutant (CPM) with big and curled petals. Glucose Phosphate Isomerase (PGI) expression in CPM mutant differed from the normal *Brassica carinata* pattern. The total glucosinolate content in CPM is higher than in APM, SSM mutants and in the original line.

INTRODUCTION

Ethiopian mustard (*Brassica carinata* Braun) is grown traditionally by Ethiopian farmers as both oilseed and vegetable crop. This species is higher yielder and tolerant to drought, pest and diseases than rapeseed under rainfeed conditions in Mediterranean climate, and could be used as a oilseed crop (Fereres et al., 1983). Like other Brassica oil crops it is characterized by the presence of high erucic acid content in its seed oil and high levels of glucosinolates in its defatted meal wich limits its utilization as edible oil and for the animal feed industry.

In 1992 we initiated a breeding programme using chemical mutagenesis aimed to the isolation of *Brassica carinata* lines with high productivity and specific seed components for both industrial and nutritional purposes i.e. (high erucic + low glucosinolate, and low erucic + low glucosinolate contents respectively).

EXPERIMENTAL

A yellow seed line, from our *Brassica carinata* collection, which had shown good performance under field was used for mutagenesis treatment with a solution of 1% (v/v) Ethyl Metane Sulfonate (EMS). The treated M1 and the consecutives mutagenized generations were grown in the field and selfpollinated.

Three types of morphological mutants were identified in M2 generation:

1. Apetalous mutant (APM) with reduced or no petals.
2. Speckled sepals mutant (SSM) with brown speckles sepals and partially opened pods.
3. Curly petals mutant (CPM) with rhomboid bud and curled petals.

The morphological mutants and the original line (OL) were analyzed for three enzyme systems and for fatty acid composition and glucosinolate content, following the methods described by Arus et al., (1991), Garcés and Mancha (1992), and Sang and Truscot (1984).

Phosphoglucoisomerase **(PGI)**

Two regions of activity, PGI-1 and PGI-2 were present. No variation was observed at PGI-1, which consisted of a single band in all the material tested. Three bands were visualized in region PGI-2. CPM mutant was the only completely distinguishable from OL. No segregations were obtained in generations M2 and M3, what indicate that PGI-2 is produced by two loci that code for monomeric subunits and the enzyme is active as a dimer. Intermediate band is resulting of the formation of intergenic heterodimers between Pgi-1 and Pgi-2.

Phosphoglucomutase **(PGM)**

Three regions of activity: PGM-3, PGM-1 and PGM-2 from the most to the least anodal were present in the zymogram of this enzyme. No variability was found at these regions in all the material evaluated. Two invariants bands were apparent in the most anodal zone. Each allele of PGM-3 is expressed by two bands. No segregation has been observed at PGM-1 in M2 and M3 generations of mutants and OL. This regions consists of three-banded pattern, what suggest that PGM-1 is controlled by two loci Pgm-1 y Pgm-1'. A single invariant band have been detected in all the individuals tested at the region PGM-2.

Leucine amino-peptidase **(LAP)**

Gels stained for LAP have two regions of activity, LAP-1 and LAP-2, with a single invariant band in the two regions for all the material analyzed.

Fatty acid composition

Table 1 summarizes the contents of the main fatty acids. The morphological mutants showed higher oleic acid and lower erucic acid content than the original line, except for the CPM-5 (in bold in Table 1) mutant wich showed a particular pattern of fatty acids: high erucic, high oleic and low linoleic and linolenic acid.

Table 1. Ranges and Mean values of fatty acid composition in morphological mutants and original line.

Fatty acids	Original line (OL)	Apetalous mutant (APM)	Speckled sepals mutant (SSP)	Curled petals mutant (CPM)
18:0	0.73-0.88	0.62-0.73	0.69-1.02	**0.54**-0.84
(%)	0.78	0.67	0.90	0.65
18:1	6.04-8.02	10.88-11.96	9.55-11.93	**11.26**-12.56
(%)	6.85	11.29	11.23	12.08
18:2	19.85-21.82	20.37-22.04	21.22-24.25	**15.44**-21.42
(%)	20.86	21.33	22.43	17.82
18:3	12.76-14.62	10.68-15.20	9.19-10.49	**8.44**-16.47
(%)	13.63	12.59	10.03	12.34
22:1	45.71-49.39	42.92-44.91	42.72-50.04	43.24-**53.96**
(%)	47.70	43.78	45.09	46.88

Glucosinolate content

All the plants analyzed showed similar glucosinolate profiles (Table 2), with sinigrin as the main glucosinolate which correlated positively with progoitrin and negatively with 4-hydroxiglucobrassicin. The glucosinolate content in CPM mutant was always higher than that of the original line. CPM-5 mutant showed the higest levels of sinigrine, progoitrine and gluconapine (Table 2, in bold).

Table 2. Ranges and Mean values of glucosinolate content (μm/g. dry matter) in morphological mutants and original line.

Glucosinolate	Original line (OL)	Apetalous mutant (APM)	Speckled sepals mutant (SSP)	Curled petals mutants (CPM)
2-hidroxy-3-butenyl (PRO)	0.5-5.8 2.65	1.2-2.6 1.85	2.9-4.3 3.62	6.7-**9.1** 8.01
Allyl- (SIN)	140.9-176.1 162.52	149.7-186.5 175.4	168.2-178.1 173.11	183.1-**201.5** 193.8
3-butenyl (GNA)	0.3-1.2 0.81	0.5-1.7 0.84	1.7-2.1 1.89	1.4-**2.9** 2.1
Benzyl- (4-OH GBS)	0.6-2.8 1.44	0.4-2.6 0.97	1.1-1.2 1.13	**0.22**-0.32 0.28
TOTAL	147.9-183.1 167.41	154.7-189.0 179.05	175.6-183.9 179.81	193.1-**213.7** 204.1

ACKNOWLEDGEMENTS

The authors thanks G. Rodriguez for technical assitance. This work has been supported by the C.I.C.Y.T. (Proj. AGF92-0225) of the Spanish Government.

REFERENCES

Arus, P., Chevre, A.M., Delourme, R., Eber, R., Kerlan, M.C., Margale, E., and Quirós, C. (1991). Isozyme nomenclature for eight enzyme systems in three Brassica species. In: *GCIRC. Eighth International Rapeseed Congress. Proceedings*. Vol. 4 . pp. 1061-1066.

Fereres, E., Domínguez, J., Mínguez, I. and Fernández-Martínez, J.M. (1983). Productivity of *Brassica juncea* and *B. carinata* in relation of rapeseed *B. napus*. *Proc. 6th. Int. Rapeseed Congr.*, Paris. France. 293-298.

Garces, R. and Mancha, M. (1992). One step lipid extraction and fatty acid methyl esters preparation from fresh tissues. *Anal. Biochemistry*. **211** : 139-143.

Sang, J.P. and Truscott, R.J.W. (1984). Liquid chromatographic determination of glucosinolates in rapeseed as desulphoglucosinolates. *J. Assoc Offic Anal Chem* **67**: 829-833.