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DEVELOPMENT OF ERUCIC ACID – AND GLUCOSINOLATE-FREE RAPESEEDS (CRUCIFERS) IN PAKISTAN*

Part II. Selection and adaptation of low Erucic Acid Crucifers in Pakistan

Shafiq Ahmad Khan, Parveen Aziz, Javed Iqbal Khan, Esar A. Butt, Khizar Hayat Khan, Lubna Salim and Abdul Waheed Sabir

PCSIR Laboratories, Lahore, Pakistan

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Low erucic acid germplasm, selected from the local as well as the imported varieties from abroad was cultiated for evaluation in Pakistan. The erucic acid levels of the parents and their generations were determined and it was found that both *B. campestris* and *B. juncea* have potentials to become erucic acid-free oilseed crops in Pakistan.

INTRODUCTION

In an earlier communication[1], the cruciferous crop of Pakistan was surveyed particularly with regard to the glucosinolate contents in the seed meal and the erucic acid in the seed oil. These studies, as reported earlier[2], were initiated with a view to selecting and introducing glucosinolate- and erucic acid-free rapeseeds in Pakistan.

The present communication presents the results obtained from the selected germplasm of the cultivated crucifers which had low erucic acid (from traces to 25%) in them. Seeds from various annual crops with low erucic acid were selected after proper evaluation and then sown under different ecological conditions for obtaining their generations. In addition, foreign cultivars claimed to be free from both erucic acid as well as glucosinolates, were also studied for adaptation and retention of low erucic acid characters in the local environments.

Selection of Local Varieties. Rapeseed crops of 1977, 1978 and 1979 were evaluated for the purpose of selecting low erucic acid germplasm from the local cultivar. Almost 2000 seed samples were scanned for determining their erucic acid contents in the oil. Out of these, about 1% seeds were selected for further propagation as they showed less than 25% erucic acid in them (Table 1). Further reduction in the erucic acid level of these selected seeds was not observed because of various factors such as non-viability of the seeds and open pollination (Table 2).

Adaptation of Introduced Crucifers. Various seeds of rape and mustard crops free from erucic acid and glucosinolate "double zero" were obtained from Canada and Sweden for their adaptation in Pakistan. These seeds belonged to *Brassica napus, Brassica Juncea, Brassica campestris* and *Brassica carinata* varieties and the erucic acid levels in their parent stock were determined before the adaptation trials (Table 3). These seeds adapted well to the Pakistani environment and showed considerable retnetion of characters in the successive generation (Table 4). Further evaluation of the stock being obtained for multiplication is under progress.

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MATERIALS AND METHODS

Collection of Samples. (a) Native cultivars: Native seed samples having low erucic acid were selected after a

Table 1. Selected lines of the local varieties with less than 25% erucic acid.

Botanical name	Code number	$C_{22:1}$%
Brassica campestris	K-676	10.86
Brassica campestris	P-13-77	16.67
Brassica campestris	K-743	19.23
Brassica juncea	S-9	12.82
Brassica juncea	L–16-75	17.05
Brassica juncea	K-488	17.27
Brassica juncea	K-61	18.19
Brassica juncea	L-6-77	20.92
Brassica juncea	L-1-77	19.08
Brassica juncea	P-488	20.93
Brassica juncea	P-43	21.21
Eruca sativa	K-805	18-81
Eruca sativa	K-740	20.90

Table 2. Erucic acid levels in successive generation of selected crucifers

Botnical name	Code number	$C_{22:1\%}$
Brassica campestris	K-676	220.20
Brassica campestris	P-13-77	Above 30
Brassica campestris	K-743	No viability
Brassica juncea	S-9	27.12
Brassica juncea	L-16-75	29.23
Brassica juncea	K-488	No viability
Brassica juncea	K-61	Above 30
Brassica juncea	L-6-77	27.39
Brassica juncea	L-1-77	20.96
Brassica juncea	P-488	No viability
Brassica juncea	P-43	10-53
Eruca sativa	K-805	No viability
Eruca sativa	K-740	No viability

Table 3. Erucic acid levels in the parent stock of the introduced crucifers

Botanical name	Cultivars	$C_{22:1\%}$
Brassica campestris	Canele	0.00
Brassica campestris	Span	4.94
Brassica carinata	SWD-4	1.58
Brassica juncea	Zem-1	0.00
Brassica juncea	Zem-2	0.43
Brassica napus	Nugget	30189
Brassica napus	Erusine	35.21
Brassica napus	Turret	34.51
Brassica napus	Target	17.26
Brassica napus	Tanka	35.80
Brassica napus	Midas	12.10
Brassica napus	Oro	14.96
Brassica napus	SWD-14	0.42

thorough evaluation from a lot of almost 2000 seed samples obtained from the open market/local farms by a survey team. Seed samples for the successive generations were supplied by the collaborating oil seed botanists. (b) Foreign cultivars: Seeds of "double zero" varieties (free from erucic acid and glucosinolates) were obtained from foreign sources through the Pakistan Agricultural Research Council (PARC) and the collaborating Institutes. Among these introduced cultivars, *'Candle,' 'Span,' 'Nugget,' 'Oro,' Tanka,'* and *Target' are of Canadian origin,* while others are mostly from Sweden. The seeds of candle variety were obtained by PARC in a lot of about 5000 tons while those of other were obtained for experimental purposes.

Erucic Acid Evaluation. Seed samples were evaluated chemically by determining their fatty acid composition through vapour phase chromatography as described below:

Determination of Fatty Acid Composition. Different seed oils were separately hydrolysed by reaction with standard alcoholic potassium hydroxide (0.5N). The soap solutions were extracted with di-ethyl ether to remove the unsaponifiable matter and then acidified with sulphuric acid (4N) to liberate the fatty acids. These fatty acids were converted into their methyl esters by the standard procedures and then resolved by vapour phase chromatography under the following conditions.

Glass column (5 ft. x 1.5 ft.), packed with DEGS (10%) injector 220°C, flame ionisation detector, 150°C, column oven, 200°C, flow rate (N_2) 40 ml/ minute (H_2) 4o ml/ minute, air 550 ml/minute.

DISCUSSION

The evaluation of the local germplasm of the crucifer cultivars shows that there is a predominant existence of erucic acid in them. It has been observed that only one per cent seeds contain less than 25% of this acid. Consequently, efforts were directed towards further reduction of this character through breeding and selection. Generation-raising procedures, however, did not give any encouraging results and it was observed that such germ-plasm as contained less erucic acid either had no viability or did not show any retention of character (Table 2).

There can be many causes for this stiuation as the generations were not raised in isolation from the common crop. The non-viability of the selected germ-plasm can also have many explanations, the most likely being immature harvesting and specific soil conditions. This was confirmed by their low oil contents (25-30%) and with 30% erucic acid and their non-viability.

Introduction of low/zero erucic acid foreign cultivars has been remarkably quick in Pakistani environment. In fact this introduction has taken place in the shortest possible time and the successive generations are clearly showing this character retention (Table 4). However, a variation in erucic acid levels is clearly discernable in case of *B. napus.* Since erucic acid occurrance is genetically controlled, reversion in case of *B. napus* is explainable as it is a cross between *B. oleracea* and *B. campestris.* [4]

Table 4. Erucic acid levels in the successive generation of the introduced crucifers

Botanical name	Cultivar	$C_{22:1}$%
Brassica campestris	Candle	3.46
Brassica campestris	Candle	T
Brassica campestris	Candle	2.64
Brassica campestris	Candle	2.13
Brassica campestris	Candle	12.55
Brassica campestris	Candle	2.0
Brassica campestris	Candle	0.71
Brassica campestris	Candle	T
Brassica campestris	Candle	10.6
Brassica campestris	Candle	0.37
Brassica campestris	Candle	0.88
Brassica campestris	Candle	1.408
Brassica campestris	SPAN	22.32
Brassica campestris	SPAN	25.10
Brassica campestris	SPAN	14.79
Brassica campestris	SPAN	2.68
Brassica campestris	SPAN	21.88
Brassica campestris	SPAN	2.65
Brassica campestris	SPAN	3.11
Brassica campestris	SPAN	22.50
Brassica campestris	SPAN	20.57
Brassica carinata	SPAN	20.57
Brassica carinata	SWD-4	T
Brassica napus	SWD-4	3.66
Brassica napus	ORO	28.20
Brassica napus	TARGET	above 30
Brassica napus	ERUSINE	above 30
Brassica napus	TURRET	25.02
Brassica napus	MIDAS	10.38
Brassica napus	MIDAS	9.37
Brassica napus	MIDAS	6.40
Brassica napus	MIDAS	37.0
Brassica napus	MIDAS	29.36
Brassica napus	MIDAS	8.72
Brassica napus	NUGGET	above 30
Brassica napus	SED-14	0.52
Brassica napus	SWD-14	0.51
Brassica napus	SWD-14	0.26
Brassica napus	SWD-14	T
Brassica napus	SWD-14	0.41
Brassica napus	SWD-14	T
Brassica napus	SWD-14	2.7
Brassica napus	SWD-14	2.5
Brassica napus	SWD-14	6.28

T = Traces

As regards the retention of characters of the foreign-introduced stock, the varieties Canlde (of *B. campestris*), SWD-4 (of *B. Carinata*), and SWD-14 (of *B. napus*) seem to be nearer to the target for achieving low erucic acid crops in Pakistan. The erucic acid range is from traces to 13.55%, trace to 3.66% and traces to 6.28% in Candle, SWD-4 and SWD-14 respectively (Table 5). The low erucic acid content in the successive generations shows that introduced varieties of crucifers have adapted to the conditions of Pakistan. The variation in the erucic acid content of the parents and the succeeding generations of Candle, SWD-4 and SWD-14 is rather small (Tables 3 and 5). It is thus expected that in further crops, lower levels of Erucic acid will be maintained and germplasm suitable for large scale cultivation will be available.

This programme for the production and development of erucic acid-free seed oil crops was based on the evaluation studies both on the field crops as well as on the wild occurring crucifers. It has been observed that low erucic acid germplasm is present in the wild flora of Paksitan. Evaluation of the wild flora, using latest technique of analysis including single cotyledon evaluation, is under study and these results will be published separately.

In future experimentation the problem of open pollination in the experimental plants will be over-come to achieve the target of low erucic acid crucifers. Genetically there is little variability in the germplasm of the introduced cultivars and it is possible to breed for low erucic acid seed lines if the contact with the higher erucic acid seed lines is

Table 5. Range of per cent variation in erucic acid of the successive generation of the introduced crucifers.

Botanical name	Cultivar	$C_{22:1}$%
Brassica campestris	Candle	T*-13.55
Brassica carinata	Span	2.65-25.10
Brassica carinata	SWD-4	T-23.66
Brassica napus	Nugget	Above 30
Brassica napus	Erusine	Avboe 30
Brassica napus	Turret	25.02
Brassica napus	Target	Above 30
Brassica napus	Tanka	Above 30
Brassica napus	Midas	2.57-37.0
Brassica napus	Oro	28.20
Brassica napus	SWD-14	T-6.28

*Traces.

checked. Controlled condition is therefore, a pre-requisite for the genetic stability and agronomic performance of any seed lines.

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