BREEDING BRASSICA NAPUS L. CANOLA WITH IMPROVED FATTY ACID COMPOSITION, HIGH OIL CONTENT AND HIGH SEED YIELD

G. Rakow[1], G.R. Stringam[2] and D.I. McGregor[1]

[1]Research Station, Agriculture Canada, 107 Science Crescent SASKATOON, Saskatchewan, S7N 0X2 CANADA

[2]Barani Agricultural Research and Development Project G.P.O. Box 1785, ISLAMABAD, Pakistan

Introduction

In Canada, breeding rapeseed with improved oil and meal quality has always had a very high priority. Market pressures have demanded superior quality oil and meal to make Canadian rapeseed and its products more competitive to other oilseeds as well as rapeseed produced in other countries. The breeding and introduction of zero erucic acid, low glucosinolate rape (B. napus L.) and turnip rape (B. campestris L.) cultivars and the complete changeover of the total Canadian rapeseed production to canola quality were important steps in establishing canola as the major Canadian oilseed. One objective in breeding to further improve oil quality has been to increase the level of linoleic acid, an essential fatty acid in human diets, and at the same time reducing the level of the easily oxidized linolenic acid. Progress towards these goals has been achieved in B. napus by several working groups throughout the world (Rakow, 1973; Röbbelen and Nitsch, 1975; Jönsson and Persson, 1983; Roy and Tarr, 1986). The world's first low linolenic acid (3%) canola quality summer rape (B. napus) cultivar Stellar, developed by Dr. Stefansson at the Plant Science Department of the University of Manitoba, Winnipeg, Canada, has recently been registered after three years of cooperative trials in Canada. This paper describes breeding work towards the development of high linoleic-low linolenic acid canola quality summer rape (B. napus) carried out at the Agriculture Canada Research Station, Saskatoon, Saskatchewan, during the last 14 years. The development of fatty acid mutants derived from mutagenic treatment of seed, their environmental stability, and the agronomic performance and fatty acid composition of lines selected from crosses between fatty acid mutants are presented.

Materials and Methods

Work towards the development of high linoleic-low linolenic acid lines of B. napus was begun in 1974. Seeds of the canola quality (0.2% erucic acid, 15 umoles/g meal glucosinolate) summer rape B. napus cultivar Tower were treated with either 0.1% ethylmethane-sulfonate (EMS), 0.008% ethyleneimine (IE), or 100 kr gamma irradiation (cobalt 60). M_1-plants were grown in the greenhouse in the winter of 1974/75, selfed and M_2 plant rows grown in the field in 1976. Open pollinated seed from five individual M_2-plants from each M_2-row was harvested and the M_3-seed analyzed for fatty acid composition. A total of approximately 1800 plants was analyzed. Seed from selected low linolenic acid plants was divided into three parts. One-third of the seed was used to advance the selection without further mutagenic treatment while half the remaining seed was

treated with 100 kr gamma rays and the other half with 0.1% EMS.

The selection scheme for the M_2 low linolenic acid selection 6697-3 is illustrated in Figure 1. Eleven low linolenic acid M_2-plants, derived from the three mutation studies, were handled in this manner.

In the winter of 1979/80 the mutants 9648 and 9650 both with normal linoleic (22%) and reduced linolenic acid (5.7%) contents (Fig. 1) were crossed with the high linoleic, slightly reduced linolenic acid mutant M11 (Röbbelen and Nitsch, 1975) in an attempt to combine low levels of linolenic of the former with high levels of linolenic acid of M11. The F_1 of this cross was grown in the field in 1980 and an F_2 nursery was grown in the field in 1981. 2,340 open pollinated F_2 plants were harvested and the seed analyzed for glucosinolate content. This was necessary since the parent M11 was high in glucosinolates. One hundred and sixty-eight low glucosinolate F_2 plants were identified by the Tes-tape method and planted into an F_3 plant-row nursery in 1982 at Saskatoon and Melfort with the cultivars Westar, Regent and Altex as checks. Agronomically superior F_3 rows were identified prior to flowering and individual plants in these rows selfed. At harvest time seed from single selfed plants was harvested individually and a bulk seed sample taken from each row. Seed from the single plants and the bulk seed samples were analyzed for fatty acid composition. Sixty-nine F_4 lines with the desired increased linoleic and decreased linolenic acid contents were yield tested in three 6-replicate tests in 1983 at Saskatoon. Two hundred and forty F_3 plants from the 1982 plant-row nursery were evaluated as F_4 lines in a plant-row nursery in 1983, and single plant selections were taken from agronomically superior rows. Eighteen superior F_4 lines were retested in a replicated yield test (Table 1) and 240 selected F_4 plants grown into an F_5 plant-row nursery in 1984. F_5 rows were assessed for agronomic characteristics, bulk harvested and the seed analyzed for oil content and fatty acid composition. Final evaluations of selected F_6 lines and of five superior F_4 lines from the 1983 and 1984 tests for agronomic performance, oil content and fatty acid composition were carried out in 1985.

Results and Discussion

a. Selection of mutants

The effectiveness of seed mutagenic treatments for the production of mutants with decreased linolenic acid contents could be demonstrated for the 0.1% EMS treatment (Fig. 1). The ethyleneimine (0.008%) and gamma ray (cobalt 60) mutagenic treatments did not produce any fatty acid mutants. The M_2 plant 6697-3 derived from the 0.1% EMS mutagenic treatment had 4.6% linolenic and a normal level of linoleic acid. The two mutant lines obtained after additional mutagenic treatments maintained their reduced linolenic acid levels in the M_4 and M_5 generations. In the M_6 generation, plant 9131-3 had an increased linoleic and normal linolenic acid content. The same pattern was found in the M_8 of this plant, but the M_9 grown under field conditions in 1981 reverted back to the mutant pattern with 23.2% linoleic and 4.9% linolenic acid. These observations indicated that single plants with linoleic/linolenic acid contents different from the original mutant genotype can maintain this modified fatty acid composition through one or two generations, and express their true genetic constitution in later generations. Similar phenomena have been reported from mutation studies aimed towards the development of high linoleic/low linolenic acid B. napus (Rakow, 1973), and were probably caused by pleiotropic gene effects. This result emphasizes the need for repeated progeny testing of fatty acid mutants in order to correctly verify their true mutant status. From the results of this study it is concluded that the three fatty acid

mutants produced have normal levels of linoleic and reduced (half normal) levels of linolenic acid. The two additional mutagenic treatments had no effects on fatty acid contents of the original mutant 6697-3. It is therefore likely that the three mutant lines carry the same mutation and are identical to each other. The mutants are similar to the mutant M57 described by Rakow (1973).

b. Environmental effects on fatty acid mutants

Levels of linoleic and linolenic acid in rapeseed varied considerably depending on the environmental conditions under which the plants were grown. Greenhouse growing conditions during the winter of 1977/78 resulted in artifically low linoleic and linolenic acid contents (Fig. 1). The 1978 field conditions favoured the synthesis of linoleic acid and its desaturation to linolenic acid, while plants of the same mutant lines grown in the field during 1979 and 1980 produced seeds with lower linoleic and linolenic acid contents, except for the plant 9131-3. This deviation is discussed above. The mutant characteristic however was present in all generations as indicated by the modified linolenic/linoleic acid ratio. This ratio was 0.20 under greenhouse conditions in the winter of 1977/78, 0.30 under field conditions in 1978, and 0.25 (excluding 9131-3) under field conditions in 1979. Zero erucic acid rape cultivars such as Westar typically produce seeds with 20% linoleic and 10% linolenic acid (Table 1) which results in a linolenic/linoleic acid ratio of 0.50.

c. Agronomic performance and oil quality of lines derived from the cross between the Saskatoon low linolenic acid mutant and the mutant M11

Seed yields of F_4 lines selected from the cross between the Saskatoon low linolenic acid mutant line and the mutant M11 were unsatisfactory, especially under favourable growing conditions when the check cultivar Westar gave a high seed yield, as in 1985 (Table 1). The oil content of the lines was also unsatisfactory at 3% lower than Westar. The lines matured later than Westar, but the difference was small and maturity would be acceptable for a first cultivar of this type. The linoleic acid contents were 8 to 9 actual percentage points higher and linolenic acid contents 2.5% lower than the respective fatty acid contents for Westar. These results indicated that the high linoleic acid trait from the mutant M11 was successfully combined with the low linolenic acid characteristic from the low linolenic acid Saskatoon mutant line through crossing and selection. The combination of these two traits in one genotype is an important achievement for further canola oil quality breeding improvements.

Low yields and oil contents of the high linoleic, low linolenic acid summer rape (B. napus) canola lines developed from the above cross prevent their immediate utilization as cultivars. In an attempt to combine the high linoleic, low linolenic oil characteristics with superior agronomic performance, these lines were crossed with and backcrossed to the high yielding well adapted summer rape (B. napus) cultivar Westar. Backcross F_2 plants were harvested in 1986 and a plant-row nursery will be sown with seeds from these plants in the summer of 1987 at Saskatoon.

References

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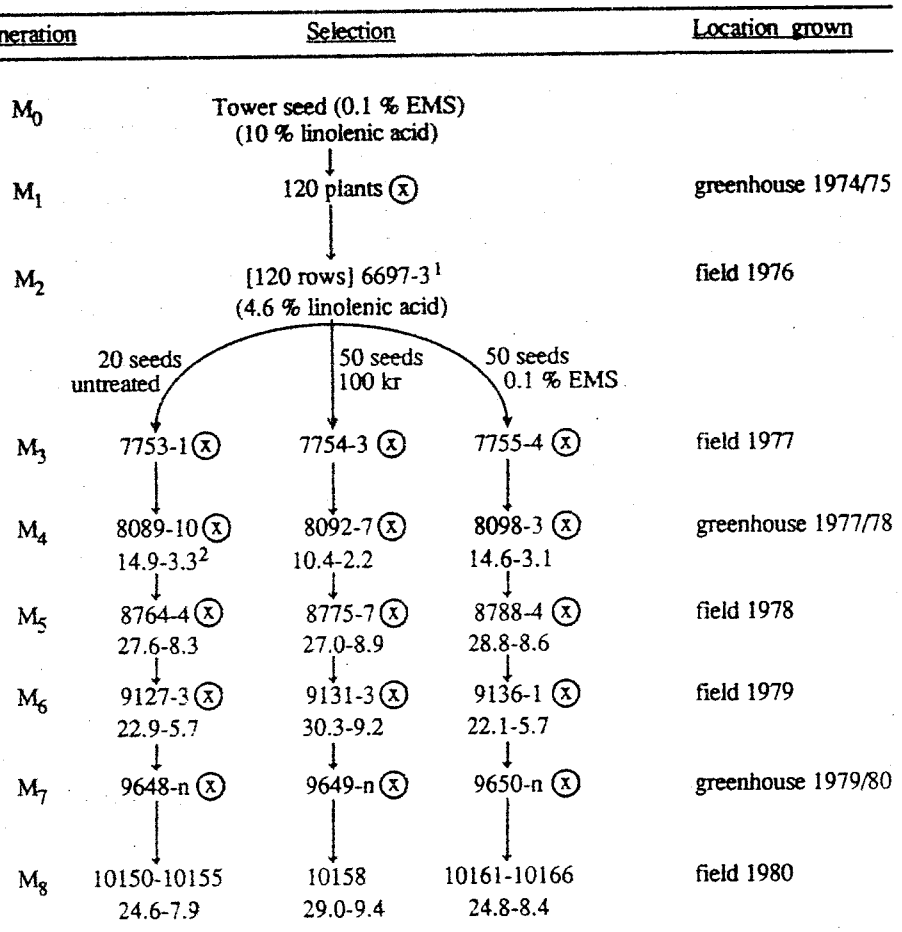
Figure 1. Pedigree of Brassica napus L. mutant lines with decreased levels of linolenic and normal levels of linoleic acid derived from the canola cultivar Tower after mutagenic treatment.



(1) 6697-3: Row number-plant number .
(2) 14.9-3.3: Percent linoleic and linolenic acid of selected plants.

Table 1: Yield, maturity, oil content, and linoleic and linolenic acid contents of 18 F_4 summer rape (B. napus) lines from the cross: Saskatoon low linolenic acid mutant X M11, relative to the cultivar Westar.

Line and Cultivar	Yield % of Westar			Difference from Westar: Maturity days			Oil %			Linoleic acid %			Linolenic acid %		
	1983	1984	1985	1983	1984	1985	1983	1984	1985	1983	1984	1985	1983	1984	1985
10275-12	98	104	64		+2	0	-3.2	-3.5	-3.0	+9.0	+7.7	+9.0	-2.4	-1.0	-2.6
10286-21-1	101	86			+1		-3.2	-3.1		+8.5	+7.3		-1.9	-1.1	
10297-17	83	95			+1		-3.3	-4.2		+8.7	+7.0		-1.9	-1.3	
10306-45	79	78	63		0	0	-3.6	-3.2	-2.7	+9.4	+7.5	+8.1	-1.5	-0.9	-2.2
Westar	2130						45.8			21.0			9.0		
10272-32	81	87		+2	-1		-0.8	-3.2		+3.9	+5.8		-1.5	-1.3	
10286-1	96	91		+1	+2		-1.4	-1.9		+7.0	+3.7		-1.9	-0.6	
10286-2-1	92	89		+4	0		-2.5	-3.4		+7.4	+6.4		-2.0	-1.0	
10286-2-2	90	92		+1	0		-1.6	-3.1		+8.1	+7.4		-2.0	-1.2	
10286-5	65	92	66	+2	0	-1	-3.4	-2.8	-2.8	+11.0	+8.2	+8.4	-1.2	-0.8	-1.7
10286-14-1	92	96	63	+1	0	0	-2.0	-3.3	-3.0	+8.8	+7.2	+7.9	-2.1	-1.3	-2.5
Westar	1582			102			43.8			21.3			8.9		
10286-14-2	80	103		+3	+1		-4.2	-3.2		+8.4	+7.5		-1.9	-1.0	
10286-16	103	94		+1	+2		-2.3	-2.7		+6.1	+5.8		-2.1	-1.0	
10286-21-2	83	94	49	0	+1	0	-3.3	-3.5	-3.0	+8.2	+7.8	+8.4	-2.5	-1.2	-2.4
10286-26	70	98		+2	+2		-2.2	-2.8		+6.3	+6.0		-1.2	-0.5	
10286-27	89	98		+1	+1		-2.8	-2.5		+9.0	+7.5		-1.5	-0.7	
10286-29	96	95		0	0		-3.3	-2.8		+7.7	+6.8		-2.5	-1.3	
10297-5-1	88	92		0	+1		-1.7	-2.2		+4.8	+4.5		-2.3	-1.2	
10297-5-2	89	88		+2	+2		-1.3	-0.7		+5.8	+4.6		-1.9	-1.0	
Westar	1563	1508	2438	102	93	98	45.7	42.8	43.8	21.8	18.3	20.5	9.1	6.0	10.1